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Increased Activity of 2',5'-Oligoadenylate Synthetase in Peripheral Blood Mononuclear Cells of Patients with Major Depressive Disorder

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The aim of this study was to measure the activity of 2',5'-oligoadenylate synthetase (2',5'AS) which is mainly induced by interferon (IFN)-α and -β in patients with major depression and evaluate its relationship to the disease. 2',5'AS activity of 23 patients (male = 11, female = 12) with major depression and of 29 normal control subjects (male = 15, female = 14) was measured in peripheral blood mononuclear cells (PBMCs) by radioassay. The mean 2',5'AS activity in the PBMCs of the patients and that of the control subjects were 1.10 ± 0.69% and 0.72 ± 0.51%, respectively. The activity in the patients was statistically higher than that of the control subjects (*P* = 0.03). These results imply some abnormality in the IFN-2',5'AS system of patients with major depression.

Key words: interferon; major depressive disorder; 2',5'-oligoadenylate synthetase

Major depressive disorder which is called major depression commonly is an agnogenic psychiatric illness whose main symptoms are disturbed drive and mood. The patient of major depression often shows loss of appetite, sleep disturbance, depressed mood, a feeling of sadness, helplessness, worthlessness and so on. Major depression and chronic fatigue syndrome (CFS) have some similar symptoms. However, it has been controversial whether they have a common pathological mechanism or not (Krusei et al., 1989; Lane et al., 1991; Terman et al., 1998). It has been reported that 2',5'-oligoadenylate synthetase (2',5'AS) activity within peripheral blood mononuclear cells (PBMCs) was elevated in patients with CFS (Suhadolnik et al., 1994; Vojdani et al., 1998; Ikuta et al., 2003). 2',5'AS is known as an interferon (IFN)-induced antiviral and antiproliferative gene product. 2',5'AS is mainly induced by IFN-α and -β, and 2',5'AS activity levels are thought to reflect the in vivo state of the IFN system (Sokawa et al., 1980; Shindo et al.,1989).

It is also known that IFN therapy for patients with type B and C hepatitis and malignant tumors induces 2',5'AS and sometimes causes serious fatigue and a depressive state. Because of these side effects, patients are treated with antidepressants, or IFN therapy is sometimes discontinued (Borden et al., 1998; Licinio et al., 1998; Valentine et al., 1998; Musselman et al., 2001). The question is whether or not and how much endogenous IFNs are involved in major depression. But it is difficult to evaluate IFN production, because cytokines including IFN are released and consumed locally at the site where the immune reaction occurs and they are

Abbreviations: AG Poly(I) Poly(C), agarose-polyriboinosinc acid-polyribocytidylic acid; 2',5'AS, 2',5'-oligoadenylate synthetase; CFS, chronic fatigue syndrome; DEAE, diethylaminoethyl; DSM-IV, Diagnostic and statistical manual of mental disorders, 4th ed.; ^{3}H-2',5'-A, ^{3}H-2',5'-oligoadenylate; HAMD, Hamilton depression rating scale; IFN, interferon; PBMC, peripheral blood mononuclear cell; PMSF, phenylmethyl sulfonyl fluoride

Table 1. Profiles and 2',5'AS activity of patients with major depression and control subjects

		Patient	Control subject	Statistics
Age	(year)	39.7 ± 10.9	37.5 ± 11.5	NS†
Sex ratio	(male/female)	11/12	15/14	NS‡
HAMD		20.8 ± 5.9	ND	
Dose of antidepressants	(mg)	79.8 ± 86.0	0	
Dose of benzodiazepines	(mg)	15.8 ± 19.3	0	
Duration of illness	(month)	82.0 ± 79.0	—	
Duration of treatment	(month)	38.4 ± 54.0	0	
2',5'AS activity	(%)	1.10 ± 0.69	0.72 ± 0.51	$P < 0.05$†

Data are mean ± SD, except the sex ratio.
ND, not determined; NS, no significant difference.
† Student's *t*-test was used for statistical analysis.
‡ χ^2 test was used for statistical analysis.

seldom detectable in peripheral blood (De Groote et al., 1992). 2',5'AS activity has been measured alternatively to IFN-α and -β activity (Sokawa et al., 1980; Schattner et al., 1981; Uno et al., 1998). IFN-α and -β in sera of patients with major depression have never been evaluated in a proper manner. The aim of this study is to measure the activity of 2',5'AS in PBMCs of patients with major depression and evaluate its relationship to the disease. This is the 1st report regarding 2',5'AS activity in patients with major depression, as far as we know.

Materials and Methods

Subjects

This study was permitted by the Medical Ethics Committee at the Tottori University Faculty of Medicine. Informed consent was obtained from every patient and healthy control subject with a detailed explanation of the purpose and goal of the study.

Eleven male and 12 female patients (mean age: 39.4 ± 10.9 years ranging from 20 to 53 years) with major depression and 15 male and 14 female healthy subjects (mean age: 37.5 ± 11.5 years ranging from 20 to 59 years) as controls participated in this study. There was no significant difference between the patient and the healthy control groups concerning age and sex ratio (Table 1). The clinical diagnosis for major depressive disorder was based on Diagnostic and statistical manual of mental disorders, 4th ed. (DSM-IV) criteria (American Psychiatric Association, 1994). Exclusion criteria included acute or chronic infection, autoimmunity, epilepsy, allergy, neoplasia, endocrine disease, CFS or other acute physical diseases for both groups. Subjects were excluded if they fulfilled any additional axis I or axis II DSM-IV diagnosis for the patients and any axis I or axis II DSM-IV diagnosis for control subjects, respectively. These illnesses were ruled out by clinical interview, physical examination and comprehensive laboratory work. For the assessment of depressive severity, psychopathology was quantified by the Hamilton depression rating scale (HAMD) 21-item version at the time of blood sampling (Hamilton, 1960). The mean HAMD score of the patients was 20.8 ± 5.9 ranging from 15 to 36. Most of them were on antidepressants such as clomipramine, maprotiline and fluvoxamine, while some were on benzodiazepines such as diazepam and flunitrazepam. The mean daily doses of antidepressants and benzodiazepins in each patient were 79.8 ± 86.0 mg (imipramine equivalents; range: 0–265 mg) and 15.8 ± 19.3 mg (diazepam equivalents; range: 0–81.5 mg), respectively. Inagaki's conversion tables for antidepressants and benzodiazepines were used (Inagaki et al., 1999). The mean durations of illness and treatment were 82.0 ± 79.0 months ranging from 1 to 324 months and 38.4 ± 54.0 months ranging from 0.3 to 192 months, respectively (Table 1).

2', 5'AS activity assay

Peripheral blood was collected between 0900 and 1200 from each subject and kept on ice. PBMCs were isolated within 30 min by Ficoll-Conray centrifugation (Boyum, 1968). The 2',5'AS activity was measured as previously described (Sokawa et al., 1980; Ikuta et al., 2003). For the assay, buffer A

[10 mM HEPES (adjusted to pH 7.5 by 1 M KOH), 3 mM magnesium acetate, 0.3 mM EDTA and 10% glycerol], buffer B (50 mM KCl in buffer A), buffer C [0.2 mM phenylmethyl sulfonyl fluoride (PMSF) and 7 mM 2-mercaptoethanol in buffer B] and buffer D (400 mM KCl in buffer A) were prepared and stocked at 4˚C until use.

The procedure of 2',5'AS activity assay was done at room temperature, if not mentioned otherwise. The 10^6 PBMCs were washed with phosphate buffered saline and were suspended in 40 μL of Triton extraction buffer (0.5% Triton X-100, 7 mM 2-mercaptoethanol and 1 mM PMSF in buffer B). The sample was mixed well by pipetting, and the cell extract of the sample was obtained by centrifugation at 14,000 rpm for 5 min at 4˚C. The cell extract was stored at –80˚C and used for the 2',5'AS activity assay within 1 week: 20 μL of the cell extract was added to 25 μL of agarose-polyriboinosinc acid-polyribocytidylic acid [AG Poly(I) Poly(C) type 6: Amersham Pharmacia Biotech, Tokyo, Japan] and put on ice for 1 h to activate 2',5'AS in the cell extract. The AG Poly(I) Poly(C) was equilibrated with buffer C before use. Then, 1 mL of buffer C was added to the mixture which contains 2',5'AS with AG Poly(I) Poly(C) and followed by centrifugation at 5,000 rpm for 1 min. The washed beads were incubated at 33˚C for 2 h with 20 μL of buffer B containing 0.5 mM cold ATP and 0.4 μCi of ^{3}H-ATP (Moravek Biochemicals, LaBrea, CA). The sample was suspended in 1 mL of buffer C and the supernatant was collected by centrifugation at 5,000 rpm for 1 min. The product [^{3}H-2',5'-oligoadenylate (^{3}H-2',5'-A)] was absorbed on 50 μL of diethylaminoethyl (DEAE) Sepharose CL-6 (Amersham Pharmacia Biotech) pre-equilibrated with buffer C. The DEAE Sepharose was washed 5 times with 1 mL of buffer C and then suspended in 500 μL of buffer D to solubilize ^{3}H-2',5'-A. The radioactivity of ^{3}H-2',5'-A in each sample was measured in a scintillator (ACS2: Amersham Pharmacia Biotech) by a scintillation counter. The 2',5'AS activity was expressed as a percentage of the incorporated ^{3}H-ATP by the enzyme in each sample. It took about 6 h from equilibration of AG Poly(I) Poly(C) to the measure of radioactivity. The intra- and inter-assay coefficients for 2',5'AS activity were 4.8% and 16.6%, respectively.

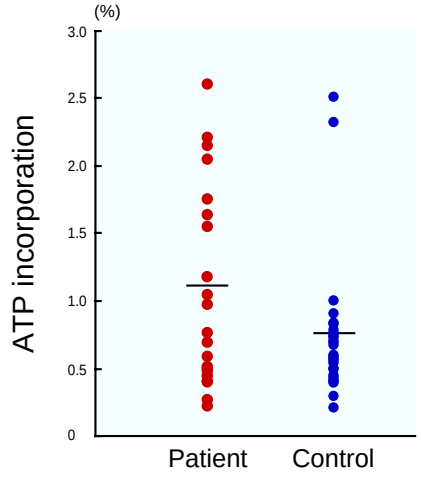

Fig. 1. The activity of 2',5'AS in PBMCs of patients with major depression and normal control subjects. Each dot shows the 2',5'AS activity for each subject. The mean 2',5'AS activity (± SD) in patients and that of the control subjects were 1.10 ± 0.69% and 0.72 ± 0.51%, respectively.

Statistical analysis

Statistical differences in age and 2',5'AS activity between the 2 groups were estimated by Student's *t*-test and that of sex distribution was estimated by the χ^2 test. Statistical difference in 2',5'AS activity between all male and female subjects was estimated by Student's *t*-test. Coefficients of 2',5'AS activities with ages, HAMD scores, dose of antidepressants, dose of benzodiazepines, duration of illness and duration of treatment were estimated by Pearson's coefficient. Differences were considered to be significant when *P* values were less than 0.05.

Results

2',5'AS activity was assayed in PBMCs of patients with major depression and healthy control subjects (Fig. 1). The mean 2',5'AS activity of ATP incorporation rate in patients with major depression and that of the healthy control subjects were 1.10 ± 0.69% and 0.72 ± 0.51%, respectively. 2',5'AS activity in the patients was statistically higher than that of the healthy control subjects ($P = 0.03$).

No significant correlation between 2',5'AS activities in PBMCs and age, HAMD scores, dose of antidepressants, dose of benzodiazepines, duration of illness or duration of treatment was observed in the patients, as well as no significant correlation between 2',5'AS activities in the PBMCs and ages in the healthy control subjects. There was also no significant sex difference of 2',5'AS activity in the PBMCs of all subjects.

Discussion

We found that 2',5'AS activity was significantly elevated in PBMCs of the patients with major depression to that of the healthy control subjects.

A depressive state is found during IFN therapy as a side effect. IFN therapy which induces 2',5'AS causes not only a central nervous system-depressive state but also somatic symptoms such as joint pain, muscle pain, fatigue and so on (Borden et al., 1998). Major depression is sometimes accompanied by those somatic symptoms, which are thought to be one of the aspects of major depression. The question was whether or not and how much endogenous IFNs are involved in major depression. IFN-α and -β in sera of patients with major depression have never been evaluated in a proper manner. Our results imply the involvement of IFN-α and -β in major depression.

Elevation of 2',5'AS activity in PBMCs of patients with CFS has been reported (Suhadolnik et al., 1994; Vojdani et al., 1998; Ikuta et al., 2003). In our study, 2',5'AS activity in PBMCs were also elevated in the patients with major depression. Even though CFS and major depression are mutually exclusive by strict diagnostic definition, there are many reports which indicate clinical overlapping or some connections between CFS and major depression (Krusei et al., 1989; Lane et al., 1991; Fukuda et al., 1994; Terman et al., 1998).

2',5'AS activity especially has been noticeable with viral infections (Schattner et al., 1981; Buffet-Janversse et al., 1983; Shindo et al., 1988). For the relation between virus and major depression, Borna disease virus antibody has been reported to be detectable within sera in patients with major depression (Bode et al., 1993). Our results may suggest the involvement of viral infections in patients with major depression.

Elevation of 2',5'AS activity in PBMCs of patients with major depression may be explained by another possibility: major depression is usually accompanied by decreased natural killer cell functions (Stein et al., 1991), and 2',5'AS might be activated as the alternative defense mechanism. The elevation of 2',5'AS activity in CFS could be a primary abnormality, because 37 kDa RNase L which is a truncated form of the native 83 kDa RNaseL is detected in patients with CFS but not in patients with major depression nor in healthy controls (Suhadolnik et al., 1997; De Meirleir et al., 2000; Demettre et al., 2002). RNase L is an enzyme which is activated with the 2',5'AS system to degrade cellular and viral single strand RNA. Thus there seems to be a different mechanism between major depression and CFS according to the elevation of 2',5'AS activity.

Most of our patients with major depression had antidepressants and benzodiazepines. Recently, it has been reported that antidepressants have a critical effect on the cytokine production of IFN-γ (Maes et al., 1999; Kubera et al., 2001). Even though we observed no significant correlation between 2',5'AS activity and those drugs in the patients, the possibility that antidepressants or benzodiazepines up-regulate 2',5'AS activity remains. In order to study the effects of those drugs on 2',5'AS activity, 2',5'AS activity in patients with major depression must be assayed before and after treatment with the drugs.

In conclusion, our study demonstrates the significant elevation of 2',5'AS activity in major depression, which implies the involvement of the IFN-2',5'AS system. The possible mechanisms of up-regulation of the enzyme remain to be studied.

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