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Levels of endocannabinoid metabolizing enzymes are not related with BDNF levels in patients with schizophrenia: a case-controlled study

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ABSTRACT

PURPOSE: While the pathogenesis of schizophrenia has yet to be fully clarified, a huge amount of data suggests the involvement of endocannabinoid system and neurotrophic factors in schizophrenia. Nevertheless, only a very limited number of studies have investigated these two systems together. With this disease containing various unknowns, our primary aim was to simultaneously investigate the serum levels of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) enzymes, which play significant roles in endocannabinoid system mechanisms, and brain-derived neurotrophic factor (BDNF) as a frequently investigated and important neurotrophic factor in patients with psychiatric disorders.
METHODS: This study comprised a total of 34 (24 men, 10 women) schizophrenia patients and 35 (26 men, 9 women) healthy control groups, aged between 18 and 65 years. PANNS and Clinical Global Impression Scale Severity of Illness (CGI-SI) were used to measure disease severity. Serum FAAH, MAGL and BDNF levels of the patients and controls were measured by conventional methods.
FINDINGS: Compared to the healthy control group, patients with schizophrenia had decreased FAAH activity, increased MAGL activity and lower BDNF levels. No correlation was noted between BDNF serum levels with FAAH or MAGL activities.
CONCLUSION: The findings of the present study showed that there were changes in the levels of metabolizing enzymes of the endocannabinoid system in schizophrenia patients, and these changes were accompanied by a decrease in BDNF levels. While this study provided important information, primarily investigating endocannabinoids and the neurotrophic factor in schizophrenia, future research should be conducted on better designed patient groups and investigate additional parameters.

Introduction

Schizophrenia is a disorder with an etiopathogenesis that is still unclear and research to address the underlying mechanisms still continues. A potential avenue of research is the endocannabinoid system. The endocannabinoid hypothesis of schizophrenia posits that overactivation of the endocannabinoid system along with disruption in the balance of dopaminergic and glutaminergic systems is associated with the symptoms of the disorder [1]. Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), which metabolize endocannabinoids, play the most significant roles in the modulation of this system [2]. Anandamide (AEA) is an endocannabinoid metabolized by FAAH, while 2-arachidonoylglycerol (2-AG) is metabolized by MAGL [3]. After they are released from neurons, endocannabinoids rapidly become inactivated by high-affinity uptake and enzymatic hydrolysis. Therefore, activity of the metabolizing enzymes may be important and affect AEA and 2-AG levels [4,5]. It will, therefore, be useful to investigate the levels of these enzymes, in order to study their relation between certain diseases and the endocannabinoid system.

Likewise, the levels of neurotrophic factors in brain have been investigated in schizophrenia patients. Neurotrophins prolong the lifespan of neurons, effect neuronal activity, axonal branching, formation of dendrites, regulation of synaptic protein levels and density of dendritic protrusions. Data on the levels of brain-derived neurotrophic factor (BDNF) in schizophrenia patients mostly indicated a decrease in these levels [6,7].

Studies performed on rats and a human study have shown that the active ingredient of cannabis modulated the expression of brain-derived neurotrophic factor (BDNF) [8,9]. These findings suggested that there could be an interaction between cannabinoids and BDNF [10]; however, no study has so far investigated the relation between BDNF and the endocannabinoid system in schizophrenia patients. This aim of this

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study was to be the first to investigate the relation between BDNF levels and the activities of FAAH and MAGL; two important enzymes, metabolizing endocannabinoids in patients with schizophrenia.

Materials and methods

Study sample

This study was carried out in the Psychiatry Department of Dicle University Training and Research Hospital from June 2016 until January 2017. A total number of 34 patients (24 men, 10 women) aged between 18 and 65 years, who were diagnosed with schizophrenia based on DSM-5 criteria, were included in the study. Before study inclusion, the diagnosis of schizophrenia was confirmed by a senior psychiatrist (MCK) through clinical interviews performed with each patient. Medical records of the patients were reviewed. Exclusion criteria were as follows: the presence of mental retardation or a cognitive function loss such as dementia, a history of or current alcohol or substance abuse (apart from tobacco), history of a severe head trauma, any new or previous chronic disease, (presence of a severe systemic disorder such as kidney and/or liver failure, epilepsy, diabetes, hypertension), comorbid psychiatric disorders or drug-related extrapyramidal symptoms; an unwillingness to participate in the study; the use of concomitant medications, other than antipsychotics or food supplements (vitamins and fish oil).

The clinical status of the patients was evaluated using the disease severity scales: Positive and Negative Symptom Scale (PANSS) and Clinical Global Impression Scale (CGI-SI). Age and gender-matched 35 (26 men, 9 women) healthy volunteers were included in the study as a control group and all the subjects in the control group underwent psychiatric interviews, as well as comprehensive neurological and physical examinations. Subjects with any psychiatric disorder or non-psychiatric medical condition were excluded from the study. In addition, subjects included in the control group had no history of a psychiatric disorder in their first-degree relatives. The study was conducted in accordance with the revised version of the Helsinki Declaration (Seul 2008) and approved by the local ethics committee (Dicle University Medical Faculty Non-Interventional Clinical Research Ethics Committee, date 13.07.2016 and number 283). Patients, their relatives and healthy subjects, were informed about the study and each provided written informed consent for participation.

Data collection tools

Sociodemographic information form

This is a semi-structured form to collect information on the sociodemographic characteristics of the patients and controls included in the study. Questions regarding age, gender, body weight, height, body mass index (BMI), marital status, level of education, occupation, level of income, duration of disease, family history of disease, any substance or drug abuse, and the quantity of cigarettes (tobacco) smoked were asked of the participants and their responses were recorded on the form.

Positive and negative symptom scale (PANSS)

This scale was developed by Kay et al., and the reliability and validity study for the scale's Turkish version was carried out by Kostakoglu et al. It is used to measure the level of positive and negative symptoms of schizophrenia, their distribution and intensity variations. It comprises 30 items in 3 sub-scales, including positive symptoms, negative symptoms and general psychopathology [11,12].

Clinical global impression scale (CGI-SI)

A 3-dimensional scale, developed by Guy, this is used to assess the severity of and treatment response in psychiatric disorders. The Clinical Global Impression-Severity of Illness (CGI-SI) dimension was used in the present study. Disease severity is evaluated on a scale of 1–7 points [13].

Biochemical analysis

Blood samples were obtained in the morning between 08.00 and 10.00 am. The samples were collected into gel tubes. Blood samples were let to clot for 15 min. Then, they were centrifuged at 5000 rpm for 6 min. Serum samples were transferred to 1.5 ml polypropylene tubes. All samples were stored at −80 centigrade degrees until the time of analysis. FAAH, MAGL and BDNF levels were measured by using human ELISA Kit (Hangzhou Eastbiopharm CO. LTD China). All measurements were obtained on the same day to minimize analysis variance, and they were repeated twice. Tests were carried out in accordance under the manufacturers' instructions. Optical intensity of each well was measured by using an automated microplate reader. The kits used for the purposes of this study were funded by the researchers.

Data analysis

All collected data were coded numerically and evaluated using the Statistical Package for Social Sciences for Windows (SPSS) version 18.0. Frequency distributions were estimated for exact statistics, and arithmetic means and standard deviation values were calculated for continuous variables. Parametric and non-parametric data were compared between two groups using the Student t-test and Mann–Whitney U test, respectively. The Tukey multiple comparison

Table 1. Demographical and clinical data of patients with schizophrenia and healthy control subjects.

Variables	Schizophrenia patients (n = 34) Mean ± SD	Controls (n = 35) Mean ± SD	Statistics		
			t	p	Cohen's d
Age (years)	36.9 ± 8.9	36.4 ± 8.0	0.27	0.782	0.059
BMI (kg/m²)	27.5 ± 3.5	27.0 ± 3.8	0.40	0.689	0.136
Cigarettes (pack years)	9.2 ± 9.6	5.3 ± 7.7	1.85	0.069	0.448
FAAH (U/L)	94.2 ± 63.1	132.6 ± 45.3	−2,91	0.005	0.699
MAGL (U/L)	133.7 ± 43.6	98.7 ± 89.9	2.04	0.045	0.495
BDNF (ng/mL)	1.01 ± 0.44	1.4 ± 0.9	−2.40	0.019	0.550
	n	n	χ2	p	
Sex (Female/ Male)	10/24	9/26	0.73	0.792	

Note: GAD: Generalized anxiety disorder, SD: Standard deviation, t: t value calculated by student t test, BMI: Body mass index, PANSS: Positive and negative syndrome scale, FAAH: Fatty acid amide hydrolase, MAGL: Monoacylglycerol lipase, BDNF: (Brain derived neurotrophic factor), N/A: Not applicable.

test was used for significant groups and the differences between groups were tested in pairs. In addition, the Pearson correlation test was used to understand the relations between different variables. The level of statistical significance was accepted as $p < 0.05$.

Results

A total number of 34 (10 women, 24 men) patients with schizophrenia and 35 (9 women, 26 men) healthy controls were included in this study. Patient and control groups did not significantly differ in terms of gender, age, BMI and smoking status (see Table 1). While serum FAAH activity and BDNF levels of schizophrenia patients were significantly lower compared to the control group ($t = −2.91$, $p = 0.005$, $t = −2.40$, $p = 0.019$), MAGL activity was significantly higher than the controls ($t = 2.04$, $p = 0.045$). Table 1 shows serum FAAH and MAGL activities and BDNF levels. While 21 patients were using a single atypical antipsychotic, 13 patients were on treatment with multiple antipsychotics. No patient was receiving monotherapy with a typical antipsychotic. When the treatment received by the patients was classified as being atypical or including multiple antipsychotics, FAAH, MAGL and BDNF levels were not found to be significantly different ($p > 0.05$). The chlorpromazine equivalent dosages of patients were calculated [14]. The mean chlorpromazine equivalent dosages were 491.0 ± 231.9 mg/day. FAAH and MAGL activities and BDNF levels were indifferent in the chlorpromazine equivalent dosages of patients ($p > 0.05$). There were no significant relations between CGI, PANSS positive (21.4 ± 7.5), PANSS negative (25.0 ± 6.5), PANSS general psychopathology (39.6 ± 10.3), PANNS total scores (85.7 ± 20.0), CGI-SI (4.8 ± 1) and disease duration (10.5 ± 6.6 years), and biochemical parameters (FAAH, MAGL activity level and BDNF level) ($p > 0.05$).

Discussion

The results of the present study demonstrated that MAGL activity was elevated, while FAAH activity was decreased and serum BDNF levels were lower in the schizophrenia patients compared to the healthy controls. On the other hand, no significant correlation was found between BDNF serum levels, and the activities of FAAH and MAGL. To the best of our knowledge, this is the first study simultaneously investigating BDNF levels and serum activities of FAAH and MAGL enzymes, which play significant roles in endocannabinoid inactivation in patients with schizophrenia.

In a study performed by Nicola De Marchi et al. regarding FAAH, the levels of mRNA encoding FAAH were measured in blood samples obtained from 12 schizophrenia patients and 20 healthy volunteers, and the levels of mRNA encoding FAAH were found to be higher [15]. The authors suggested that the decrease in FAAH was compensation for increased AEA levels. During the same study, they showed that the levels of mRNA encoding FAAH decreased after treatment given to only 5 patients. They associated the decrease in FAAH with the use of antipsychotic medications. In the present study, FAAH activity was found to be lower in patients whose schizophrenia was under control, compared with the control group. Nevertheless, although the study performed by Nicola De Marchi et al. was important as a follow-up study, this study was limited, as they had an insufficient number of cases and did not investigate MAGL levels. In another study, Bioque M et al. showed increased FAAH expression in peripheral macrophages of patients with the first-episode psychosis ($n = 95$) compared with healthy controls [16]. As mentioned above, this decrease noted in our patients may also be due to indirect compensation of the psychotic signs through AEA and antipsychotic effects. The same study also showed that MAGL expression was enhanced in peripheral macrophages in the patient group compared with the healthy controls. Both 2-AG and metabolizing MAGL play significant roles in inflammation. In rat models of brain disorders, MAGL inhibition showed anti-inflammatory and neuroprotective effects [17]. Our data indicated an increase in MAGL activity. This could be associated with increased inflammation, one of the mechanisms considered to be involved in etiopathogenesis of schizophrenia [18]. In addition, some studies have demonstrated that FAAH inhibition induces anti-inflammatory effects [19,20]. There are

also some studies suggesting that AEA protects neurons [21]. Therefore, in our study, while the decrease in FAAH may have contributed positively, the increase in MAGL could have alleviated inflammation in schizophrenia patients. Impairment of this balance may present a subject of research in pathogenesis.

In another study, the levels of endocannabinoids in CSF (cerebrospinal fluid) were compared between 10 schizophrenia patients (five treatment naïve, two had not used any antipsychotics for the previous 7 days and three patients using antipsychotics; four of those patients had a history of substance abuse) and 11 healthy controls. While AEA levels of these patients were found to be elevated, a decrease was noted in 2-AG levels [22]. The levels of FAAH, which metabolizes AEA, decreased while the levels of MAGL metabolizing 2-AG increased in the present study, a finding that is consistent with earlier literature. *In vivo* studies indicated that the interaction of D2 with AEA was more prominent compared to 2-AG. As Leweke FM stated, anandamide is released in the rat brain upon the activation of D2 dopamine receptors. It was therefore suggested that the endogenous cannabinoid system might reflect a homeostatic adaptation to dopamine imbalances [22–24]. Our findings also supported this hypothesis. Alternatively, increased anandamide concentrations in CSF may reflect a primary *hypercannabinergic* state, which can develop in the presence of schizophrenia or a subgroup of schizophrenic syndromes [25,26]. These elevations may play a role in disease pathogenesis through factors altering enzyme activity.

In a study including 47 schizophrenia patients, 84 healthy controls, 13 patients with dementia and 22 patients with affective disorders, the AEA levels of schizophrenia patients who were not using antipsychotics were found to be higher compared with healthy controls, and patients with dementia or affective disorder [27]. The increase in AEA levels as demonstrated in that study is in line with our data indicating a lower FAAH activity in schizophrenia patients. The fact that the findings of this study were different in schizophrenia patients compared with the other psychiatric disorders indicates the need for a detailed investigation of the endocannabinoid system in schizophrenia.

ECS is suggested to play a role as a neuroprotective system that is activated in the presence of certain neurodegenerative and neuroinflammatory injuries [28,29]. Synthesis of endocannabinoids was suggested to be a defense mechanism adopted by the brain during a psychotic state [16,27,30]. In mice models, endocannabinoids were found to show neuroprotective effects by increasing BDNF during epileptic seizures . In a review published by Hwang J et al. in 2010, FAAH inhibition was shown to be neuroprotective in several in vitro and in vivo studies, as well as against several neuropathological conditions including Alzheimer,

Huntington and Parkinson diseases, stroke and traumatic brain damage [31]. The low levels of BDNF found in this study may indicate that the pathology still continues in the presence of schizophrenia and FAAH activity may be reduced to accelerate this process. However, one may also argue that the decrease in BDNF might be insufficient and MAGL elevation can indirectly enhance the inflammation.

Limitations of this study include its cross-sectional design, heterogeneous distribution of the medications used by our patients, absence of the measurements of endogenous cannabinoids and cannabinoid synthesizing enzymes, a wide age range of controls and patients, and limited sample size.

In conclusion, our results have indicated that in patients with schizophrenia there is an imbalance in endocannabinoid system enzymes and BDNF levels are decreased. We did not find any significant correlation between these two systems. Our findings could play a significant role in future research on schizophrenia. Large-scale studies with longer duration of follow-up are required to investigate additional endocannabinoid parameters in conjunction with tropic factors in schizophrenia patients.

Disclosure statement

No potential conflict of interest was reported by the authors.

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