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Journal

Journal of Psychopharmacology, 22(3)

ISSN

0269-8811

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Publication Date

2008-05-01

DOI

10.1177/0269881107083816

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Peer reviewed

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Journal of Psychopharmacology
22(3) (2008) 262–269
© 2008 British Association
for Psychopharmacology
ISSN 0269-8811
SAGE Publications
Los Angeles, London,
New Delhi and Singapore
10.1177/0269881107083816

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Abstract

Disturbances in the endogenous cannabinoid (ECB) system in schizophrenia may contribute to their enhanced sensitivity to psychoactive substances, and the beneficial effects of second-generation antipsychotics for substance abuse in schizophrenia may involve modulatory effects on ECB. To verify these two assumptions, 29 patients (24 completers) with schizophrenia and substance use disorders (SUD) were treated with quetiapine for 12 weeks, and peripheral ECB levels were measured, using high-performance liquid chromatography/mass spectrometry, in patients (weeks 0, 6 and 12) and 17 healthy volunteers. Baseline anandamide levels were significantly higher in patients, relative

to controls. This result is consistent with studies describing ECB dysfunctions in schizophrenia. SUD parameters improved during treatment, but no changes in ECB occurred over time. Improvements in substance abuse were probably not mediated by modulatory effects of quetiapine on ECB. Lastly, baseline anandamide predicted endpoint SUD scores (alcohol/ cannabis). Anandamide is a potential target for medications aimed at relieving SUD in schizophrenia.

Keywords

schizophrenia, substance use disorders, cannabis, endogenous cannabinoids, anandamide, quetiapine

Introduction

There is a growing interest for the link between schizophrenia and substance abuse. The discovery of the endogenous cannabinoid (ECB) system may help to understand the nature of this link. For decades, cannabis has been reported to induce a transient psychotic disorder, which mimics the positive symptoms of schizophrenia

(Nunez and Gurpegui, 2002). Cannabis also seems to provoke an amotivational syndrome mimicking the negative symptoms of the disorder (Solowij and Grenyer, 2002). In addition, cannabis disrupts cognitive functions (e.g., selective attention) recognized as being impaired in schizophrenia (Emrich *et al.*, 1997; Pope *et al.*, 1995; Pope *et al.*, 2001). Moreover, recent well-controlled studies suggest that cannabis smoking is associated with a two to three-fold

increase in psychotic symptoms, but not necessarily in psychosis diagnoses (Arseneault *et al.*, 2002; Semple *et al.*, 2005). In light of these observations, cannabis has recently emerged as a reliable 'model psychosis' (D'Souza *et al.*, 2004).

Discovered in the last decade, the ECB system is composed of at least two natural ligands, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and at least two cannabinoid receptors (CB₁ and CB₂) (Ameri, 1999). Preliminary data suggest that the ECB system is dysfunctional in schizophrenia. CB₁ receptors are distributed in high densities in brain regions known to be impaired in schizophrenia, such as the prefrontal cortex, hippocampus and basal ganglia (Ameri, 1999). Moreover, AEA levels are abnormally elevated in the cerebrospinal fluid (CSF) of schizophrenia patients (Leweke *et al.*, 1999; Giuffrida *et al.*, 2004). Also, post-mortem studies have shown altered CB₁-receptor densities in schizophrenia (Dean *et al.*, 2001). Lastly, an association between the CB₁-receptor gene polymorphism and schizophrenia may exist (Leroy *et al.*, 2001).

The heightened vulnerability of schizophrenia patients to psychoactive substances (PAS), including cannabis, is largely acknowledged. The odds ratio of lifetime substance abuse in patients with schizophrenia is two or three times higher than in the general population – for cannabis, it is five or six times higher (Regier *et al.*, 1990). Also, the frequency of psychotic relapses and hospitalizations is increased in schizophrenia patients who abuse PAS, including cannabis (Linszen *et al.*, 1994). However, the reasons for this heightened vulnerability remain unclear. One explanation may lie in biological disturbances common to both schizophrenia and substance abuse. For instance, some authors have proposed that dopamine sensitization, a well-characterized pathophysiological feature of schizophrenia (Moghaddam and Krystal, 2003), may render patients more sensitive to the rewarding effects of PAS (Chambers and Self, 2002; Tsapakis *et al.*, 2003). Similarly, we recently proposed that disturbances in the ECB system may provide a mechanistic explanation for the high prevalence of substance use disorders (SUD) in schizophrenia patients (Potvin *et al.*, 2005), considering the implication of the ECB system in both psychotic and addictive processes (Ameri, 1999; Giuffrida *et al.*, 2004; Arnold, 2005).

The pharmacological treatment of substance abuse in schizophrenia has not attracted systematic interest until recently. So far, the most promising results have been obtained with clozapine (Green *et al.*, 2002). Based on current knowledge no antipsychotic drugs have affinities for CB₁ receptors. However, Sundram *et al.* (2005) have recently shown that chronic administration of clozapine, but not haloperidol nor chlorpromazine, decreases [³H]CP55940 binding to the CB₁ receptor in the rat nucleus accumbens. This brain region receives dopaminergic projections from the ventral tegmental area and mediates the rewarding effects of most PAS, including cannabis (Wise, 2002). This finding has led this group to hypothesize that the beneficial effects of clozapine for SUD in schizophrenia may involve modulatory effects on the ECB system (Sundram *et al.*, 2005).

Quetiapine shares crucial pharmacological properties with clozapine: fast dissociation from D₂-dopamine receptors, similar 5-HT_{2A}/D₂-affinity ratio and partial agonism at 5-HT_{1A} serotonin

receptors (Meltzer *et al.*, 2003). The modulatory effects of quetiapine on CB₁ receptors are unknown. But benefits similar to those of clozapine have been reported with quetiapine in schizophrenia patients, abusing amphetamines, cocaine or cannabis (for a review, see Potvin *et al.*, 2005). Based on these preliminary results, we conducted an open-label trial investigating the impact of quetiapine in dual diagnosis schizophrenia. During the trial, we sought to determine whether: (i) baseline ECB predicts substance abuse evolution in patients during treatment and (ii) quetiapine modulates plasma ECB, as these modulatory effects may provide mechanistic explanations for the reported benefits of second-generation antipsychotics in dual diagnosis patients.

Methods

Participants

Patients were diagnosed with a schizophrenia spectrum disorder and a comorbid SUD (abuse or dependence) (last three months), using the Structured Clinical Interview for DSM-IV. All patients signed a detailed informed consent form. The study was approved by the local scientific and ethics committee, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Exclusion criteria were the following: (i) patients already on quetiapine or clozapine; (ii) patients hospitalized or acutely ill; (iii) patients in need of an inpatient addiction treatment program; (iv) total score lower than 65 on the Positive and Negative Syndrome Scale (PANSS) (Kay *et al.*, 1987); (v) pregnancy; (vi) female subjects of childbearing potential without adequate contraception; (vii) abnormal liver function (hepatic enzymes more than three times the upper normal limits) and (viii) any clinically meaningful unstable renal, hepatic, cardiovascular, respiratory, cerebrovascular disease or other serious, progressive physical disease.

Thirty-three dual diagnosis outpatients were screened; four patients did not meet our inclusion criteria. At baseline, 29 patients were switched from their previous antipsychotic medication(s) to quetiapine for a 12-week open-label trial. Twenty-three patients completed the whole trial, and another one completed nine weeks of the study. There were five cases of dropouts for the following reasons: lost-to-follow-up (two patients), dissociative experience (one patient), heightened hostility (one patient) and tachycardia (one patient).

The 29 patients assessed on baseline suffered from schizophrenia ($n = 16$), schizoaffective disorder ($n = 11$) and schizophreniform disorder ($n = 2$). Mean duration of illness was 91.3 ± 97.4 months. Mean education level was 11.0 ± 2.1 years. Patients were diagnosed with one or more of the following SUD (abuse or dependence): cannabis (18 patients), alcohol (13 patients), cocaine (7 patients), amphetamine (1 patient), hallucinogens (1 patient) and phencyclidine (PCP) (1 patient). SUD diagnoses were complemented with drug urine screenings.

Controls consisted of 17 healthy volunteers. Patients and controls were similar in terms of age [patients: 30.1 (mean) ± 10.1 years (SD) versus controls: 26.9 ± 6.0 years; $t = 1.218$; $P = 0.230$], sex (patients: 25 males and 4 females versus controls: 14 males and

3 females; $\chi^2 = 0.123$; $P = 0.725$) and ethnicity (patients: 27 Caucasian versus controls: 15 Caucasian; $\chi^2 = 0.320$; $P = 0.572$). There were no significant differences of weight between patients and controls (patients: 82.4 ± 24.5 kg versus controls: 74.1 ± 15.4 kg; $t = 1.259$; $P = 0.215$).

Before being switched to quetiapine, study completers ($n = 24$) were treated with one or more of the following antipsychotics: olanzapine (15 patients), risperidone (5 patients), ziprasidone (1 patient), haloperidol (3 patients) and other conventional antipsychotics (4 patients). Dosage of quetiapine (between 200 and 800 mg) and titration followed the guidelines specified in the Canadian Product Monograph, 2000. Compliance to quetiapine was assessed via pill count, complemented with information from the family, pharmacy and/or social worker. Mean prescribed dose of quetiapine (week 12) for trial completers ($n = 24$) was 545.8 ± 258.2 mg, whereas the mean taken dose was 466.6 ± 227.3 mg (pill count). Concomitant drugs were allowed. Participants received the following adjuvant medications: antidepressants (eight patients), mood stabilizers (five patients), anxiolytics (three patients) and anticonvulsants (one patient).

Assessments

To measure SUD in patients with schizophrenia, several instruments were administered on weeks 0 (baseline) and 12 (endpoint) (for more information, see Potvin *et al.*, 2006). Quantities used (last week) were registered, according to the TimeLine Follow-Back procedure (Sobell and Sobell, 1992). Quantities taken were registered for all PAS, not only the patient's drug of choice. Amount spent on drugs of abuse was calculated based on the market value in Quebec province (Canada). SUD severity was measured with the Alcohol and Drug Use Scales (AUS and DUS) (Drake *et al.*, 1990). The AUS and DUS are five-point scales based on the DSM-IV criteria for severity of SUD: 1 = abstinence; 2 = use without impairment; 3 = abuse; 4 = dependence and 5 = severe dependence. SUD severity (all PAS) was also evaluated with an eight-item adapted scale, based on the DSM-IV criteria of substance dependence. The patients' SUD severity was scored [from 0 (no problem) to 5 (severe problem)] on the following items: (i) loss of control, (ii) time spent on PAS, (iii) impact of SUD on social life, (iv) impact of SUD on daily occupations, (v) physical impact of SUD, (vi) psychiatric impact of SUD, (vii) impact of SUD on compliance and (viii) ability to enjoy pleasures other than substance use.

Psychiatric variables were assessed with the PANSS and the Calgary Depression Scale for Schizophrenia (CDSS) (Addington *et al.*, 1990) at baseline, weeks 6 and 12. For psychiatric and substance abuse variables, raters were not blind with respect to repeated measures.

ECB/bioactive fatty-acid ethanolamides

Peripheral AEA and 2-AG were measured in dual diagnosis patients and healthy volunteers. As exploratory analyses, palmitylethanolamide (PEA) and oleylethanolamide (OEA) were also measured. PEA has well-documented anti-inflammatory actions (Lo Verme *et al.*, 2005), whereas OEA is an anorexic lipid

that produces satiety and reduces weight gain in rodents (Fu *et al.*, 2003). Although structurally related to AEA, PEA and OEA are two non-cannabinoid bioactive fatty-acid ethanolamides (FAEA), as these lipids do not bind to cannabinoids receptors, but bind with high affinity to the peroxisome-proliferator-activated receptor-alpha (PPAR- α) (Fu *et al.*, 2003; LoVerme *et al.*, 2005).

We collected blood samples (2 mL) between 13:00 and 16:00 pm, using heparinized tubes. Within 1 h, blood samples were centrifuged (3200 rpm for 15 min), and plasma (1 mL) was stored at -80°C in glass vials. Plasma samples were spiked with internal standards (100 pmol of [$^2\text{H}_4$]-OEA and [$^2\text{H}_4$]-PEA, 10 pmol of [$^2\text{H}_4$]-AEA and 250 pmol of [$^2\text{H}_8$]-2-AG), and proteins were precipitated by adding cold acetone. Lipids were extracted with chloroform/methanol/water and quantified by isotope-dilution (Giuffrida *et al.*, 2000) using a 1100-liquid chromatography system coupled to a 1946A-mass spectrometer detector (Agilent Technologies, Inc., Palo Alto, CA) equipped with an electrospray ionization chamber. Lowest limits of quantification were 0.1 pmol for AEA, 1.2 pmol for OEA and PEA and 1.8 pmol for 2-AG.

Statistical analyses

Differences in ECB/FAEA plasma levels between patients and controls were assessed by analysis of variance (ANOVA) with group as the independent variable. Evolution of psychiatric symptoms, substance abuse and ECB/FAEA during quetiapine treatment was assessed for significance using ANOVA for repeated measures. The Kolmogorov-Smirnov one-sample test for normality was applied. Among FAEA, OEA displayed a non-normal distribution ($Z = 1.589$; $P = 0.013$). For OEA, non-parametric tests were thus used (e.g., Mann-Whitney and Wilcoxon tests). Simple linear regression analyses were also performed, using baseline AEA as a predictor and endpoint psychiatric and substance use scores as dependent variables. Outlier data (Q -test for heterogeneity) were replaced by mean scores. Statistical analyses were conducted with the Statistical Package for Social Sciences, version 10. The critical level of significance for rejecting the null hypothesis was set at 5%.

Results

Clinical evolution (psychiatric symptoms and SUD)

Overall, among patient completers, SUD severity scores (composite AUS-DUS, DSM-IV adapted scale) improved during the study. A decrease in dollars spent weekly on PAS (all PAS) was observed (for more information, see Potvin *et al.*, 2006). Improvements in positive, negative and depressive symptoms were also observed (Table 1).

ECB in patients and controls

At baseline, AEA was detectable in 28 patients (out of 29) and 2-AG in 23 patients. AEA was detectable in 17 healthy volunteers (out of 17) and 2-AG in eight controls. At baseline, AEA levels were significantly elevated in dual diagnosis patients, compared

Table 1 Symptomatic and substance abuse evolution in time (ANOVA for repeated measures)

Measures	Week 0		Week 6		Week 12		Baseline versus quetiapine		
	Means	SEM	Means	SEM	Means	SEM	F	DF	P
PAS use (in dollars)	93.2	13.2	60.2	13.6	62.4	12.2	6.927	1,46	<0.05
Alcohol (\$)	30.7	7.4	20.0	5.6	23.5	6.4	2.274	1,42	NS
Cannabis (\$)	53.2	13.4	38.1	10.0	47.3	13.3	1.886	1,36	NS
DSM-IV adapted scale*	22.1	0.9	18.1	1.1	17.3	1.6	11.738	1,46	<0.01
AUS	2.5	0.2	–	–	2.3	0.2	1.683	1,23	NS
DUS	3.4	0.3	–	–	3.0	0.3	4.832	1,23	<0.05
PANSS-positive	18.5	0.9	17.1	0.7	15.9	0.9	10.46	1,46	<0.01
PANSS-negative	19.4	1.0	18	1.0	15.8	0.9	8.259	1,46	<0.01
PANSS-general	41.8	1.2	39.8	1.3	36.3	1.4	8.766	1,46	<0.01
PANSS-total	79.8	2.2	75.1	2.4	68	2.7	12.33	1,46	<0.01
CDSS	7.3	1.0	6.8	1.1	4.1	0.8	8.628	1,46	<0.01

*Scores range from 0 to 40.

SEM, standard error of the mean.

with healthy volunteers, but not 2-AG levels (Table 2). Despite improvements in substance abuse, AEA levels remained significantly elevated in patients, relative to controls, after treatment with quetiapine [AEA: $F(1,39) = 6.29$; $P = 0.017$; 2-AG: $F(1,26) = 2.31$; $P = 0.141$]. Also of interest, AEA levels were found to be elevated in a sub-group of seven schizophrenia patients who showed a marked reduction in substance use at study endpoint (score ≤ 2 on the AUS and DUS scales), relative to controls (AEA levels = 6.9 ± 1.7 pmol; $F_{lin} = 4.369$; $P = 0.043$).

Evolution of ECB in time

Among patient completers, no significant changes in AEA and 2-AG plasma levels were observed during quetiapine therapy (Table 3).

Table 2 Baseline endogenous cannabinoids in patients with patients with schizophrenia and substance use disorder, relative to controls

	Patients (baseline)		Controls		F	DF	P
	Mean (pmol)	SEM	Mean	SEM			
ECB							
AEA	6.3	1.2	2.1	0.3	7.130	1,44	0.011
2-AG	39.4	6.1	22.5	3.2	2.150	1,30	0.153
FAEA							
PEA	13.0	1.7	5.7	1.7	6.163	1,23	0.021
					U^*		
OEA	22.1	5.5	4.5	0.4	63.000		0.0001

*Mann-Whitney U .

Simple linear regression analyses

Simple linear regression analyses were performed using AEA and 2-AG as predictors, and substance use scores and psychiatric symptoms as dependent variables. To increase statistical power, these analyses involved study completers ($n = 24$) and dropouts ($n = 5$). As shown in Table 4, baseline peripheral AEA of patient completers was a significant predictor of endpoint PAS use (in dollars) [$F(1,26) = 13.50$; $P = 0.001$], alcohol drinks [$F(1,26) = 4.41$; $P = 0.046$], AUS scores [$F(1,26) = 5.39$; $P = 0.028$], cannabis joints [$F(1,26) = 7.97$; $P = 0.009$] and DSM-IV adapted scale score [$F(1,26) = 6.53$; $P = 0.017$]. Baseline 2-AG did not predict endpoint substance abuse scores, baseline AEA and 2-AG did not predict endpoint psychiatric symptoms, and baseline psychiatric and substance use scores did not predict endpoint AEA and 2-AG values.

Exploratory analyses

As exploratory analyses, peripheral PEA and OEA levels were also measured. Elevated PEA levels have been described in schizophrenia (Leweke *et al.*, 1999). Here, we sought to determine whether PEA levels are also elevated in dual diagnosis patients and whether this abnormal functioning can be modified by quetiapine. As for OEA, it was of interest to explore its potential relationships with weight problems in schizophrenia (Müller *et al.*, 2004) and weight gain associated with quetiapine (Newcomer, 2005).

At baseline, PEA was detectable in 16 patients and OEA in 29 patients. PEA was detectable in eight controls and OEA in 17 controls. At baseline, PEA and OEA plasma levels were significantly elevated in dual diagnosis patients, compared with healthy volunteers (Table 2). Despite improvements in substance abuse, PEA and OEA levels remained significantly elevated in patients, relative to controls, after treatment with quetiapine [PEA: $F(1,20) = 4.91$; $P = 0.039$; OEA: $U = 60.50$; $P = 0.0001$].

Table 3 Endogenous cannabinoids during quetiapine therapy

	Baseline		Week 6		Week 12		F	DF	P
	Mean (pmol)	SEM	Mean	SEM	Mean	SEM			
ECB									
AEA	5.8	1.1	6.6	1.6	5.7	1.2	0.126	2,44	0.882
2-AG	40.9	7.1	32.2	5.6	34.8	4.6	0.708	2,38	0.499
FAEA									
PEA	13.2	1.8	11.9	1.0	11.7	1.7	0.335	2,26	0.718
							Weeks	Z*	
OEA	22.0	6.3	34.7	9.5	20.5	4.1	0 to 6	-0.900	0.368
							0 to 12	-0.400	0.689

*Wilcoxon Z.

Table 4 Baseline endogenous cannabinoids as predictors of substance abuse evolution in dual diagnosis schizophrenia (simple linear regression analyses)

Dependent variable	Predictor	R ²	β	t	P
Endpoint PAS use (in dollars)	AEA*	0.342	0.585	3.674	0.001
Endpoint alcohol drinks	AEA	0.145	0.381	2.101	0.046
Endpoint AUS	AEA	0.172	0.414	2.322	0.028
Endpoint cannabis joints	AEA	0.235	0.484	2.822	0.009
Endpoint DSM-IV adapted scale	AEA	0.201	0.448	2.554	0.017

*n = 28.

β, standardized regression coefficient; R², % of the variance of the dependent variable explained by the predictor.

Among patient completers, no significant changes in PEA and OEA plasma levels occurred during quetiapine therapy (Table 3).

Discussion

Table 1 summarizes the results for psychiatric symptoms and substance abuse (for more information, see Potvin *et al.*, 2006). Overall, the improvements in substance abuse observed during the trial are consistent with preliminary results, showing benefits of second-generation antipsychotics in dual diagnosis patients (Green *et al.*, 2002; Brown *et al.*, 2003). During the trial, we sought to determine whether: (i) baseline ECB predicts substance abuse evolution in patients during treatment and (ii) quetiapine modulates plasma ECB, as these modulatory effects may provide mechanistic explanations for the reported benefits of second-generation antipsychotics in dual diagnosis patients.

Endogenous cannabinoid

At baseline, peripheral levels of AEA were increased in dual diagnosis patients, compared with healthy volunteers. This result is consistent

with the study of De Marchi *et al.* (2003), who found elevated plasma AEA levels in schizophrenia patients. It is also consistent with the findings of Giuffrida *et al.* (2004) and Leweke *et al.* (1999) in the CSF of patients, but discrepant with the results from the same group, who did not find increased plasma AEA levels in schizophrenia patients (Giuffrida *et al.*, 2004). The most studied ECB, AEA, is a full CB₁ agonist and a partial agonist at CB₂ receptors (Gonsiorek *et al.*, 2000). AEA has multiple functions in the central nervous system (CNS), including processing of natural and drug rewards, memory, stress and pain relief (Ameri, 1999). In the present study, the increased peripheral AEA levels may reflect a state of arousal, produced by an activation of the peripheral sympathetic system. Consistent with this interpretation, physical exercise has been recently shown to produce an elevation in plasma AEA levels (Sparling *et al.*, 2003).

To our knowledge, this is the first study to measure 2-AG in patients with schizophrenia. There was no significant difference in baseline 2-AG in patients when compared with controls. This result does not support the hypothesis of Pryor (2000), who proposed an excess in 2-AG platelet release as a mediator of cognitive deficits in schizophrenia.

In par with one of our hypotheses, we discovered that baseline AEA levels were significant predictors of endpoint substance abuse

scores. Higher AEA levels on baseline predicted worse substance abuse outcomes (cannabis and alcohol) at study endpoint. This result is consistent with pre-clinical studies showing a role for AEA in drug addiction (Arnold, 2005; Basavarajappa and Hungund, 2002; Ameri, 1999), and it may suggest that disturbances in the ECB system in schizophrenia contribute to their enhanced sensitivity to cannabis and alcohol.

Contrary to our expectation, no changes in ECB levels were observed during quetiapine therapy. Therefore, it is unlikely that the presumed benefits of quetiapine in dual diagnosis patients depend on modulatory effects on ECB. This lack of effect of quetiapine contrasts with the pre-clinical findings from Sundram *et al.* (2005) using clozapine; with the results of De Marchi *et al.* (2003), who showed a decrease in peripheral AEA levels in schizophrenia patients responding to olanzapine treatment; and with the finding from Giuffrida *et al.* (2004), who showed cross-sectional differences in cerebrospinal AEA levels in schizophrenia patients treated with first- and second-generation antipsychotics. This lack of effect of quetiapine on ECB levels might suggest that the elevated ECB levels in dual diagnosis patients are trait- rather than state-dependent. This lack of effect of quetiapine must be interpreted cautiously. First, schizophrenia patients in this study were also substance abusers. Despite significant reductions in substance use in time, we cannot exclude that changes in peripheral ECB may have occurred after prolonged abstinence. Second, it must be noted that the patients in this study were stable in terms of psychiatric symptoms at baseline, whereas patients in the trial from De Marchi *et al.* (2003) were acutely ill, and changes in ECB levels were calculated only for those patients who achieved remission during treatment (5 out of 20 patients). In addition, patients were mostly on second-generation antipsychotics at baseline in this study. In the De Marchi *et al.* (2003), patients were free of medication (>30 days), whereas Giuffrida *et al.* (2004) compared the effects on the ECB system of second-generation with those of first-generation antipsychotics. Lastly, it must be considered that peripheral ECB levels may not reflect brain ECB function. Thus, we cannot rule out an effect of quetiapine on brain ECB function based on our negative findings in the periphery. Noteworthy, Giuffrida *et al.* (2004) found differences between first- and second-generation antipsychotics only for ECB measured in the CSF, not for peripheral ECB.

Exploratory analyses

At baseline, peripheral levels of PEA and OEA were increased in patients, compared with healthy volunteers. PEA and OEA levels remained elevated in patients at study endpoint, as no significant changes in PEA and OEA levels occurred during quetiapine therapy.

PEA has well-documented anti-inflammatory actions (Lo Verme *et al.*, 2005). Therefore, the increased PEA levels found in patients may be related to inflammatory processes associated with schizophrenia (Garver *et al.*, 2003) and/or drug abuse (Szabo, 1999).

OEA is an anorexic lipid that produces satiety and reduces weight gain in rodents (Fu *et al.*, 2003). Here, we found increased plasma OEA levels in patients, relative to controls. This result is

counter-intuitive, since patients with schizophrenia tend to be overweighted (Müller *et al.*, 2004), not the reverse. However, patients in this sample did not significantly differ in weight compared with controls. This paradoxical result may be due to substance abuse (including cannabis), which is often associated with loss of appetite (Falck-Ytter and McCullough, 2000; Budney *et al.*, 2004). As for the lack of changes in OEA levels during quetiapine therapy, it must be considered in light of the lack of weight gain observed during the study (baseline: 79.3 ± 18.1 kg (SD); end-point: 79.1 ± 17.6 kg; $F(1,23) = 0.024$; NS). Further studies are required about the potential role of OEA in appetite/weight disturbances in schizophrenia patients and/or substance abusers.

Scope and summary

The observed elevations in AEA levels at baseline are in keeping with growing literature, describing ECB dysfunctions in schizophrenia. Schizophrenia is thought to involve impairments in dopamine, glutamate and acetylcholine (Moghaddam and Krystal, 2003). Interestingly, these neurotransmitters stimulate AEA synthesis/release in rodents (Giuffrida *et al.*, 1999; Stella and Piomelli, 2001). The fact that baseline AEA levels predicted substance abuse outcomes is of direct interest for future biological studies on dual diagnosis schizophrenia. This finding may suggest that disturbances in the ECB system in schizophrenia contribute to their enhanced sensitivity to cannabis and alcohol. Also, the predictive value of AEA makes it a potential target for new pharmacological probes aimed at relieving substance abuse in patients with or without schizophrenia. Three pharmacological strategies have been examined in pre-clinical studies to modulate AEA activity: (i) the blockade of CB₁ receptors; (ii) the inhibition of AEA transport and (iii) the inhibition of fatty acid amid hydrolase (FAAH), an enzyme that catalyzes AEA (Cravatt and Lichtman, 2003). In humans, rimonabant (a CB₁ antagonist) reduced the acute effects of cannabis (Huestis *et al.*, 2001) and is currently under investigation for tobacco smoking in a phase-III clinical trial. In rodents, rimonabant has been shown to reduce alcohol preference (Lallemand *et al.*, 2001), heroin self-administration (De Vries *et al.*, 2003) and cocaine reinstatement (De Vries *et al.*, 2001). As for AM404 (AEA transport inhibitor), it seems to relieve the signs of withdrawal of morphine in rodents (Del Arco *et al.*, 2002).

This study had some limitations. Overall, the results cannot be attributed *per se* to the pharmacological effects of quetiapine, for three main reasons: (i) the open-label design of the study, which does not control the placebo effect; (ii) the patients' poor compliance and (iii) the fact that patients were closely monitored. Second, the size of the sample may explain some of our negative results, such as the negative result for 2-AG. This result may reflect a type-II error, as 2-AG was detectable in 23 patients and 8 controls. Moreover, drugs of abuse were heterogeneous (mostly alcohol, cannabis and cocaine). However, increasing pre-clinical studies show a modulatory role of the ECB system in alcohol-related behaviors (Basavarajappa and Hungund, 2002; Colombo *et al.*, 2005). In addition, the peripheral measurement of ECB makes it difficult to draw conclusions about CNS functioning. Lastly, this study lacked a comparison group of non-abusing schizophrenia

patients, which precluded from determining whether elevations in AEA at baseline were related to schizophrenia, substance abuse or an interaction between the two conditions. This problem will be addressed by our group in the near future. However, it must be considered that AEA remained elevated in patients relative to controls at study endpoint, despite significant reductions in PAS consumption during the trial. Importantly, AEA levels were also found to be elevated in a sub-group of patients who were not substance abusers anymore at study endpoint, relative to controls. These facts make it unlikely that AEA elevations were merely the result of the acute effects of PAS.

In summary, an increase of baseline AEA levels was observed in patients with schizophrenia and SUD, compared with controls. The result of increased peripheral AEA levels in patients are in keeping with a growing amount of evidence, linking disturbances in the ECB system to the pathophysiology of schizophrenia. Contrary to our expectation, no significant changes in ECB levels were observed during quetiapine treatment. It is thus unlikely that improvements in substance abuse were mediated by modulatory effects of quetiapine on ECB. Lastly, baseline AEA predicted substance abuse outcomes, which makes it a potential target for pharmacological agents aimed at relieving substance abuse in schizophrenia.

Acknowledgements

The authors wish to dedicate this work to Dr Jean-Yves Roy, a pioneer psychiatrist in Quebec who passed away before the completion of this trial. The study – an Investigator Initiated Trial – was supported by AstraZeneca Pharmaceuticals. The experiments complied with the current laws of Canada. ES is a holder of the Eli Lilly Chair of Schizophrenia from the University of Montreal. SP is a holder of a doctoral scholarship from the Canadian Institute in Health Research (CIHR). The authors would like to thank the doctors, students and staff who helped in recruitment (Pierre Léouffre, Christo Todorov), patient follow-up (Patrick Barabé, Richard Cloutier, Mimi Dumont, Lise Fournier, Marc-André Sirois) and study assessments (Nancy Légaré, Tania Pampoulova).

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