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Relationship between dopamine D_2 receptor occupancy, clinical response, and drug and monoamine metabolites levels in plasma and cerebrospinal fluid. A pilot study in patients suffering from first-episode schizophrenia treated with quetiapine

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ABSTRACT

Combining measurements of the monoamine metabolites in the cerebrospinal fluid (CSF) and neuroimaging can increase efficiency of drug discovery for treatment of brain disorders. To address this question, we examined five drug-naïve patients suffering from schizophrenic disorder. Patients were assessed clinically, using the Positive and Negative Syndrome Scale (PANSS): at baseline and then at weekly intervals. Plasma and CSF levels of quetiapine and norquetiapine as well CSF 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole-acetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were obtained at baseline and again after at least a 4 week medication trail with 600 mg/day quetiapine. CSF monoamine metabolites levels were compared with dopamine D_2 receptor occupancy (DA-D_2) using [^{18}F]fallypride and positron emission tomography (PET). Quetiapine produced preferential occupancy of parietal cortex *vs.* putamenal DA-D_2, 41.4% ($p < 0.05$, corrected for multiple comparisons). DA-D_2 receptor occupancies in the occipital and parietal cortex were correlated with CSF quetiapine and norquetiapine levels ($p < 0.01$ and $p < 0.05$, respectively). CSF monoamine metabolites were significantly increased after treatment and correlated with regional receptor occupancies in the putamen [DOPAC: ($p < 0.01$) and HVA: ($p < 0.05$)], caudate nucleus [HVA: ($p < 0.01$)], thalamus [MHPG: ($p < 0.05$)] and in the temporal cortex [HVA: ($p < 0.05$) and 5-HIAA: ($p < 0.05$)]. This suggests that CSF monoamine metabolites levels reflect the effects of quetiapine treatment on neurotransmitters *in vivo* and indicates that monitoring plasma and CSF quetiapine and norquetiapine levels may be of clinical relevance.

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1. Introduction

Quetiapine is a dibenzothiazepine that exhibits properties of a second generation antipsychotics (Goldstein, 1995). The prediction of atypicality is based on the pharmacological profile of the compound, which seems to be similar to that of clozapine (Chiodo and Bunney, 1983; Goldstein et al., 1993; Meltzer, 1992). The atypicality of quetiapine is mainly a consequence of antagonism of the serotonergic 5-HT_{2A} receptors, which leads to disinhibition of dopamine and noradrenaline release, that is dopaminergic and noradrenergic signaling in the mesocortical pathway (Shayegan and Stahl, 2004).

Chronic treatment with quetiapine produces depolarization inactivation of ventral tegmental DA neurons, sparing those in the substantia nigra, whereas haloperidol inactivates both (Chiodo and Bunney, 1983; Goldstein et al., 1993). Although quetiapine binds to multiple CNS neurotransmitter receptors (Schotte et al., 1996), the above studies suggest that its atypical profile may be mediated, at least in part, by preferential effects on dopamine (DA) D_2 receptor-mediated neurotransmission in cortex and limbic regions, compared to the dorsal striatum.

Quetiapine is rapidly absorbed after oral administration (t_{max}: 1–1.5 h; C_{max} after 25 mg quetiapine: 53–117 ng/ml) but also rapidly eliminated ($t_{1/2}$: 3.1–5.5 h) (DeVane and Nemeroff, 2001). Among its metabolites, norquetiapine displays high affinity for 5-HT_{2A} receptors and therefore, it may contribute to the antipsychotic activity of quetiapine. In addition, norquetiapine is probably an antidepressant, by its high affinity for the norepinephrine reuptake site and for the 5-HT_{1A} receptor (Jensen et al., 2008). Dose corrected steady-state trough concentrations of quetiapine in plasma are highly variable (Gerlach et al., 2007; Hasselstrøm and Linnet, 2004; DeVane and Nemeroff, 2001). Very little is known about the pharmacokinetic properties of norquetiapine. In steady-state conditions, its peak (2 h) levels are considerably lower than those of its parent compound, after administration of 400 mg/day quetiapine (Winter et al., 2008). It is generally considered that drug CSF better than plasma concentrations reflect its fate in the brain (Bianchine and McConnell, 1994; DeLange and Danhof, 2002). Therefore, in this study, both quetiapine and norquetiapine levels in plasma and CSF were compared with D_2-occupancy in brain and with their clinical activity.

One way to examine the DA, serotonin (5-HT) and noradrenaline (NA) systems in the brain is through measurement of their metabolites homovanillic acid (HVA), 5-hydroxyindolacetic acid (5-HIAA), and 3-methoxy-4-hydroxyphenylglycol (MHPG), in cerebrospinal fluid (CSF). Several studies with typical antipsychotics (Garelis and Sourkes, 1973; Potter and Manji, 1993; Weir et al., 1973) have demonstrated that levels of HVA, 5-HIAA and MHPG in CSF reliably reflect their levels in the brain, implying that they actually reflect central DA, 5-HT and NA turnover and thus can be used to examine the effects of antipsychotic drugs *in vivo* (Agnati et al., 1995; Silver et al., 1996). However, studies examining the effects of the second generation antipsychotics on CSF monoamine metabolites in schizophrenia are lacking (Scheepers et al., 2004).

To evaluate quetiapine binding to DA-D_2 receptor in extrastriatal regions, we used PET with [^{18}F]fallypride (Kessler et al., 2000; Mukherjee et al., 2002) to measure the levels of DA-D_2 receptor occupancy in putamen, caudate nucleus, thalamus, temporal cortex, parietal cortex and occipital cortex in schizophrenic patients who were treated with quetiapine for 4 weeks. [^{18}F]fallypride is a high-affinity radioligand for DA-D_2 and D_3 receptors that can be used to quantitate levels of DA-$D_{2/3}$ receptors in man in both striatal and extrastriatal regions with a single tracer injection (Siessmeier et al., 2005; Stark et al., 2007).

Previously, two groups of authors already demonstrated in their PET studies occupation of DA-D_2 receptors by quetiapine, using either [C^{11}]raclopride or [^{18}F]fallypride as ligands (Kessler et al., 2006; Kapur et al., 2000), but quetiapine was only measured in plasma. In the present study, the relationship between DA-D_2 receptor occupancy, and the quetiapine and norquetiapine and monoamine metabolites levels in plasma and CSF was investigated immediately before injection of the radiotracer [^{18}F]fallypride.

2. Patients and methods

The study was approved by the local ethics committee in Frankfurt a.M., Germany, and the German radiation safety authorities. During the study all patients were inpatients at the Klinikum Fulda gAG, Department of Psychiatry and Psychotherapy, Fulda. All PET investigations were performed at the Department of Nuclear Medicine, Klinikum Fulda gAG, Fulda, Germany.

2.1. Patients

Five drug-naïve male schizophrenic patients in their first episode who fulfilled the criteria for schizophrenia according to the DSM-IV (APA, 2004) were included after giving written informed consent. The mean ± SEM age was 34.4 ± 4.4 years (age range; 25–46 years). To determine clinical outcome, each patient was rated on the Positive and Negative Syndrome Scale (PANSS) for severity of illness and for improvement with treatment (Kay et al., 1987). All patients were treated with quetiapine 600 mg/day for 4 weeks.

2.2. Radiochemistry

The [^{18}F]fallypride was synthesized by a novel high-yield modification of the method for the synthesis of [^{18}F]desmethoxyfallypride (Gründer et al., 2003). In brief, the tosylated precursor ((S)-*N*-[(1-allyl)-2-pyrrolidinyl)-methyl]-5-(3-toluenesulfonyloxy-propyl)-2,3-methoxybenzamide (5 mg, 10 μmol) was dissolved in 1 mL acetonitrile, treated for 5 min at 65 °C with potassium carbonate (5 mg, 36 μmol), and subsequently transferred into a 5 mL vial containing [^{18}F]fluoride using the method of Hamacher et al. (1986). The reaction mixture was heated for 20 min at 85 °C, diluted with 1 mL phosphoric acid (10%), and separated using high-performance liquid chromatography (HPLC) (250 × 10 mm, RP8; CH_3CN: 0.25 mol/L ammonium acetate buffer + 5 mL acetic acid/L, 30:70; 5 mL/min). The fraction containing [^{18}F]fallypride was isolated, diluted with 0.15 mol/L disodium hydrogen phosphate buffer, and adsorbed on a C_{18} cartridge to remove the HPLC solvent. The column was washed with 2 mL water and the product was eluted with 1 mL ethanol. The eluant was diluted with 9 mL of an isotonic sodium chloride solution and sterilized by filtration (0.22 μm). Quality control before injection included determination of the chemical and radiochemical purity, specific activity, pH, and absence of pyrogens.

2.3. Data acquisition and analysis

Images were acquired on a GE Advance whole body PET scanner. Data acquisition comprised of a series of 38 time frames [5 min transmission scan followed by injection of [^{18}F]fallypride; 10 × 1 min, 6 × 5, (15 min pause), repositioning, 8 × 5, (15 min pause) and a final 8 × 5 min] with total scan duration of 165 min. A mean of 327 ± 94 MBq (mean ± SD) [^{18}F]fallypride was injected intravenously as a bolus. Measured binding potentials (BP) were calculated on a voxelwise basis using the Lammertsma Simplified Reference Tissue Model, which is based on a two-tissue compart-

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ment model (Lammertsma and Hume, 1996). The cerebellum was chosen as a reference region, since it is generally considered dopamine receptor free. We cannot exclude the possibility that the occupancy values in our study are slightly underestimated due to a very small specific binding in the cerebellum (Mukherjee et al., 2001). PET imaging was performed before (drug-naïve) and after 4 weeks of quetiapine therapy.

2.4. Calculation of DA-D_2 receptor occupancy

Receptor occupancy was defined as percentage reduction of BP relative to the baseline BP according to the following equation: Receptor occupancy $(\%) = [(1) - (BP_{medicated}/BP_{unmedicated}) \times 100]$. A fixed ROI template was defined to evaluate BP values from individual studies on the stereotactically normalized images using patients own magnetic resonance imaging (MRI) scans comprising the following areas: putamen, caudate nucleus, thalamus, temporal cortex, parietal cortex and occipital cortex.

2.5. Statistical analyses

To evaluate whether quetiapine produced selective and/or non-uniform occupancy of DA-D_2 receptors in extrastriatal brain regions compared to the putamen, an analysis of variance for regional DA-D_2 receptor occupancy with region as a factor and covaried for the putamen occupancy was performed. *Bonferroni* corrections were used to adjust significance levels for multiple comparisons. Baseline and after treatment CSF monoamine metabolites and DA-D_2 receptor-BP values were compared using two-tailed probability *t* tests for paired samples. The change in clinical indices was assessed using a repeated-measures analysis of variance. Correlations of DA-D_2 receptor occupancy with plasma and CSF quetiapine and norquetiapine levels and with CSF monoamine metabolites were performed using *Pearson* product moment correlations (*r*).

As a nominal level of significance, $\alpha < 0.05$ was accepted. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 17.0.

2.6. Sample collection and assay techniques

Baseline CSF samples for the analysis HVA, 5-HIAA, MHPG and DOPAC, were collected at 9:30 a.m., immediately before [^{18}F]fallypride bolus injection. Thereafter, the baseline PET scans were started between 10:00 a.m. and 10:30 a.m. The same procedure was rerun on day 28. The full daily dose of quetiapine was administered at 8:00 a.m. At 9:30 a.m., whole blood was collected for monitoring of quetiapine and norquetiapine. Lumbar puncture (LP) was performed directly after blood was obtained and CSF collected for determination of monoamine metabolites and quetiapine and norquetiapine.

Quetiapine and norquetiapine in human ethylenediaminetetraacetic acid (K_2EDTA) plasma and CSF were assessed by BASi (West Lafayette, Indiana). Plasma and CSF concentrations of quetiapine and norquetiapine were determined in human plasma and human CSF using a method of liquid–liquid extraction, followed by reversed-phase liquid chromatography separation and electrospray ionization tandem mass spectrometry detection (LC/MS–MS). Quetiapine and norquetiapine labeled internal standards were extracted from K_2EDTA human plasma and human CSF, the organic layer collected, evaporated to dryness, and the residue reconstituted with an ammonium formate buffer. The reconstituted samples were injected into an LC/MS–MS system using a gradient ammonium formate/organic mobile phase, with a quantitation range from <0.7 ng/mL to at least 2000 ng/mL for each analyte (Davis et al., 2010).

CSF levels of HVA, 5-HIAA, MHPG and DOPAC were determined by reverse-phase high-performance liquid chromatography, as previously described (Little et al., 1999; Stenfors et al., 1995; Weikop et al., 2007).

3. Results

3.1. Drug levels and DA-D_2 receptor occupancy

Plasma and CSF levels of quetiapine and norquetiapine are displayed in Table 1. In all patients, plasma and CSF levels of quetiapine were higher than those of norquetiapine, with a mean quetiapine/norquetiapine ratio of 3.05 and 4.47 [(range: 0.96–6.69) and (0.43–15.47)] in plasma and CSF, respectively. Mean plasma and CSF quetiapine and norquetiapine levels differed at the time of the scans with ([^{18}F]fallypride (Table 1). No correlations were found between the plasma and CSF quetiapine and norquetiapine levels.

There were no correlations between putamen, caudate nucleus, thalamus, temporal cortex, parietal cortex and occipital cortex DA-D_2 receptor occupancy and plasma quetiapine and norquetiapine levels (Table 2). DA-D_2 receptor occupancies correlated to CSF quetiapine and norquetiapine levels in the occipital cortex ($p < 0.01$) and in the parietal cortex ($p < 0.05$).

Drug-naïve schizophrenic patients had significantly higher mean DA-D_2 receptor-BP values at baseline than after treatment in the putamen (11.1 ± 1.1, (mean ± SEM), *vs.* 7.6 ± 1.1; $t_{1,4} = 4.16$; $p = 0.014$), caudate nucleus (10.4 ± 1.2 *vs.* 7.7 ± 1.5; $t_{1,4} = 5.89$; $p = 0.004$), thalamus (2.6 ± 1.4 *vs.* 1.9 ± 1.2; $t_{1,4} = 3.32$; $p = 0.029$), temporal cortex (0.3 ± 0.04 *vs.* 0.2 ± 0.01; $t_{1,4} = 2.98$; $p = 0.048$), parietal cortex (0.2 ± 0.04 *vs.* 0.1 ± 0.02; $t_{1,4} = 4.88$; $p = 0.008$), and occipital cortex (0.3 ± 0.04 *vs.* 0.2 ± 0.02; $t_{1,4} = 3.57$; $p = 0.023$).

Table 1
Plasma and CSF quetiapine and norquetiapine levels and receptor occupancies in several brain regions.

Patients ($n = 5$)	Quetiapine (ng/mL)		Norquetiapine (ng/mL)		D_2[a] receptor occupancy (%)					
	[^{18}F]fallypride[b]		[^{18}F]fallypride[b]		Individual occupancy values in several brain regions					
	Plasma	CSF[c]	Plasma	CSF[c]	Putamen	Caudate nucl.	Thalamus	Temporal cortex	Parietal cortex	Occipital cortex
1	1610	50	149	6	14	12	17	38	34	45
2	507	4	181	4	17	32	28	14	44	21
3	127	7	132	4	60	56	63	61	55	25
4	1740	20	260	6	37	33	38	24	36	24
5	1410	56	444	4	31	14	26	31	38	42
Mean ± SEM	1078.8 ± 321.1	27.5 ± 10.8	233.2 ± 57.1	4.1 ± 0.2	31.8 ± 8.2	29.4 ± 8.1	34.4 ± 7.8	33.6 ± 7.9	41.4 ± 3.8	31.4 ± 5.0

[a] D_2 indicate type 2 dopamine receptors.
[b] Determined immediately before injection of the radiotracer [^{18}F]fallypride.
[c] CSF: Cerebrospinal fluid.

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Table 2
Pearson product moment correlations for the relationship between receptor occupancies, plasma and CSF quetiapine and norquetiapine and CSF monoamine metabolites levels.

After treatment	Plasma (ng/mL)		CSF[a] (ng/mL)		CSF[a] monoamine metabolites (nmol/L)			
Brain regions	Quetiapine	Norquetiapine	Quetiapine	Norquetiapine	DOPAC[b]	HVA[c]	5-HIAA[d]	MHPG[e]
Putamen	−0.65	−0.04	−0.35	−0.31	**0.98****	**0.88***	0.47	0.41
Caudate nucl.	−0.54	−0.36	−0.46	0.10	0.82	**0.97****	0.62	0.79
Thalamus	−0.66	−0.31	−0.64	−0.16	0.82	0.16	0.38	**0.89***
Temporal cortex	−0.39	−0.32	0.01	0.15	0.76	**0.88***	**0.91***	0.38
Parietal cortex	−0.79	−0.16	−0.61	**−0.94***	0.11	0.08	−0.57	0.10
Occipital cortex	0.56	0.36	**0.96****	0.34	−0.14	−0.31	0.44	−0.60

[a] CSF: Cerebrospinal fluid.
[b] DOPAC: 3,4-dihydroxyphenylacetic acid.
[c] HVA: homovanillic acid.
[d] 5-HIAA: 5-hydroxyindolacetic acid.
[e] MHPG: 3-methoxy-4-hydroxyphenylglycol.
* $p < 0.05$.
** $p < 0.01$.

An analysis of variance for DA-D_2 receptor occupancy in quetiapine-treated patients was performed using region as a factor and covaried for putamen occupancy (Table 1 and Fig. 1). DA-D_2 receptor occupancy was higher in the parietal cortex ($p = 0.018$). No significant difference in occupancy compared with putamen was found for the caudate nucleus, thalamus, temporal and the occipital cortex.

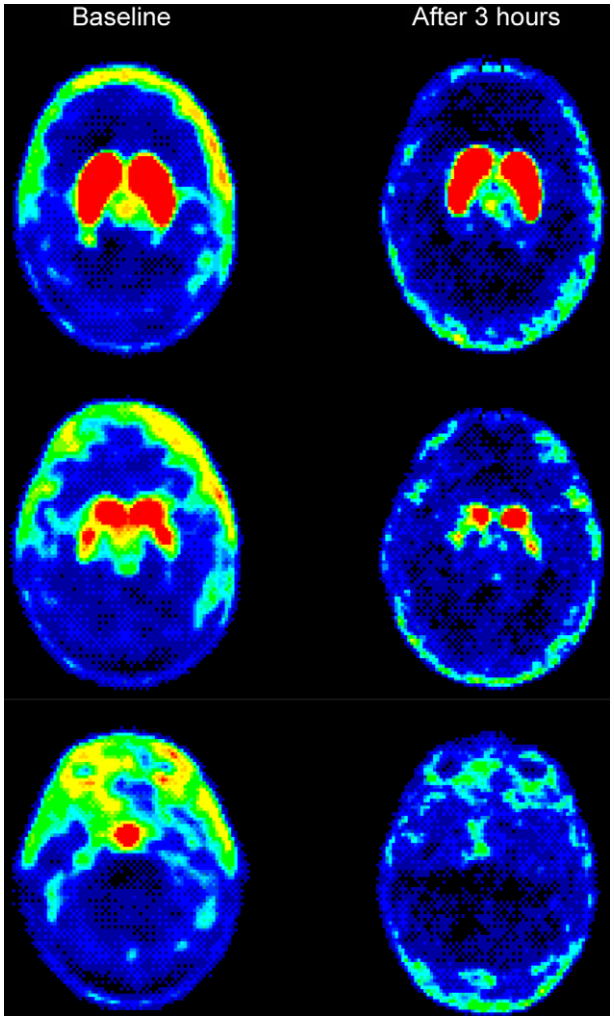

Fig. 1. Positron emission tomography (PET) scans using [^{18}F]fallypride in atypical patient at the beginning of treatment and 3 h after the last dose (600 mg/day for 4 weeks).

3.2. CSF monoamine metabolites and receptor occupancy

Quetiapine administration was associated with a significant increase (mean ± SEM) in CSF HVA from 129.2 nmol/L ± 21.0 to 184.2 ± 43.1 nmol/L ($t_{1,4} = 3.32$, $p = 0.012$); 5-HIAA: 67.3 nmol/L ± 11.2 to 87.4 ± 8.9 nmol/L ($t_{1,4} = 8.45$, $p = 0.001$); MPHG: 29.2 nmol/L ± 2.9 to 97.8 ± 5.3 nmol/L ($t_{1,4} = 10.30$, $p < 0.001$); DOPAC: 15.0 nmol/L ± 3.6 to 19.4 ± 3.2 nmol/L ($t_{1,4} = 5.01$, $p = 0.007$).

CSF levels of DOPAC and HVA (Table 2) correlated with the putamen DA-D_2 occupancy ($p < 0.01$) and ($p < 0.05$); HVA levels correlated with the caudate nucleus occupancy and the temporal cortex DA-D_2 occupancy ($p < 0.01$ and $p < 0.05$). 5-HIAA levels correlated with the temporal cortex occupancy ($p < 0.05$) and MHPG levels showed a significant correlation with the DA-D_2 occupancy in the thalamus ($p < 0.05$).

3.3. Clinical assessment and outcome

Treatment with quetiapine resulted in significant improvement in clinical symptoms on the following parameters: PANSS total score [mean ± SEM (range)]: baseline: 94.6 ± 3.2 (88–106); week four: 49.2 ± 6.2 (38–72); ($t_{1,4} = 11.36$; $p = 0.001$). Repeated-measures ANOVA showed for all schizophrenic patients a main effect of time, suggesting a pronounced reduction in the PANSS subscales: PANSS total score: ($F_{4,4} = 220.2$, $p = 0.001$); positive subscore: ($F_{4,4} = 62.9$, $p = 0.001$); negative subscore: ($F_{4,4} = 22.3$, $p = 0.009$); general psychopathology subscore: ($F_{4,4} = 98.4$, $p = 0.001$) after 4 weeks of quetiapine treatment.

4. Discussion

To the best of our knowledge the present study is the first to evaluate effects of quetiapine treatment by measuring plasma and CSF levels of quetiapine and norquetiapine and correlating them to the clinical outcome and changes in CSF levels of monoamine metabolites immediately before injection of the radiotracer [^{18}F]fallypride.

We examined five drug-naïve patients suffering from schizophrenia that were treated for four weeks with 600 mg/day quetiapine. DOPAC, HVA, 5-HIAA and MHPG in the CSF were measured at baseline and at the end of the experimental period. Clinical assessments were obtained at weekly intervals.

This study has several limitations, including small sample size, absence of a placebo group, and possible confounding by stress associated with the lumbar puncture and hospital confinement.

The major findings of this study are: (1) significant clinical improvement was achieved at DA-D_2 receptor occupancies that

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were in all examined regions lower than the 65–70% threshold seen with typical antipsychotics (Kapur et al., 1996; Nordström et al., 1993). Moreover, the putamen DA-D_2 occupancies for quetiapine were lower than those reported for [^{11}C]raclopride PET (Farde et al., 1994; Gefvert et al., 1998; Tauscher-Wisniewski et al., 2002), (2) DA-D_2 receptor occupancy by quetiapine was higher in the parietal lobe than in the putamen, and (3) quetiapine significantly increased CSF monoamine metabolites concentrations; the increase correlated significantly with the regional receptor occupancies in the putamen, caudate nucleus, thalamus, and the temporal cortex.

The first PET study with quetiapine (Kapur et al., 2000) cannot be compared with the present investigation or with that of Kessler et al. (2006), as they measured DA receptor occupancy in striatum only and 12 h after the last quetiapine dose. Due to background noise, even negative occupancy was measured for the lowest dose (150 mg/day quetiapine) while the highest dose (600 mg/day) resulted in only 19% occupancy due to the rapid elimination of quetiapine (cf. Section 1). Interestingly, in steady-state conditions, 12 h after the last dose, norquetiapine plasma concentrations are higher than those of its parent compound (Winter et al., 2008), but norquetiapine also has low affinity for DA receptors, and drug kinetics in brain are not linearly related to those in blood.

Recently, Kessler et al. (2006) studied the occupancy of putamenal, ventral striatal, thalamic, amygdala, substantia nigra, and temporal cortical DA-D_2 receptors using PET with [^{18}F]fallypride in schizophrenic patients treated with either clozapine or quetiapine. They found limited evidence for the preferential occupancy of DA-D_2 receptors in cortex and limbic regions. Both clozapine and quetiapine produced lower levels of putamenal DA-D_2 receptors occupancy (47.8% and 33.5%, respectively) than those reported for typical antipsychotic drugs. In particular, quetiapine also produced preferential occupancy of temporal cortical DA-D_2 receptors (46.9%), but did not spare occupancy of substantia nigra DA-D_2 receptors.

In the present study, DA-D_2 receptors occupancy was significantly higher ($p = 0.018$) only in the parietal cortex compared to that in the putamen (Table 1). Parietal and temporal cortex apparently did not react in the same way; this result could be a consequence either of low receptor occupancies in extrastriatal regions or could not be shown due to the small number of patients. A direct comparison between the study of Kessler et al. (2006) with the present investigation is limited by the fact that in their study, all but one patient were treated with lower quetiapine doses (200–400 mg/day), but in both their and our study, PET analysis was performed 2 h after the last quetiapine dose.

Using [123I]epidepride SPECT, Pilowsky et al. (1997) demonstrated a higher occupancy of temporal cortical than striatal D_2 receptors by clozapine. This group reported the same observation for a number of other atypical antipsychotics (Bigliani et al., 2000; Bressan et al., 2003).

Determination of CSF monoamine metabolites and obtaining [^{18}F]fallypride PET in the same patients at baseline and at the end of the experimental period allowed us to examine the interaction between monoaminergic transmission and neuronal activity, as reflected by striatal and extrastriatal DA-D_2 receptor occupancy of neurons receiving monoaminergic input. The positive relationships between CSF monoamine metabolites and striatal and extrastriatal DA-D_2 receptor occupancy (Table 2) may indicate an inhibitory influence of dopaminergic, noradrenergic and serotonergic pathways on neurons in the cortical and subcortical terminal brain areas.

It was expected that the pharmacological effect of quetiapine (and its metabolites) on the CSF concentrations of neurotransmitters would be in some relationship with the pharmacokinetics of quetiapine and norquetiapine in CSF and plasma of the patients, but this could not be demonstrated. Moreover, no significant correlation could be reported between plasma quetiapine and DA-D_2 receptor occupancy, but only between CSF drug concentration and DA-D_2 receptor occupancy in the occipital cortex ($p < 0.001$; Table 2). It has to be considered that quetiapine is an atypical antipsychotic drug with particular pharmacokinetic properties, which may explain some of these puzzling observations. After quetiapine administration, relatively short t_{max} (1–1.5 h) and $t_{1/2}$ (3.1–5.5 h) were measured in plasma of patients (DeVane and Nemeroff, 2001). In the present study, these properties were considered in the preparation protocol, in that blood and CSF were drawn 1.5 h after the last full dose of quetiapine, but 1–1.5 h before DA-D_2 receptor occupancy was measured by PET. However, due to the rapid absorption and elimination of the drug, it was quite possible that in some patients, blood and CSF were drawn in the absorption phase and in others in the elimination phase, while PET was carried out in the steep elimination phase.

In conclusion, this study demonstrates the importance of measuring levels of a drug and its metabolite in blood and CSF together with changes in putative biomarkers of the disease. The combination of measurements of the monoamine metabolites in the CSF with imaging techniques constitutes an innovative *in vivo* strategy to facilitate drug discovery and develop programs to test whether a drug engages its intended target.

Conflict of interest statement

The authors agree with the submission of the present manuscript and have no conflicts of interests related to publication of this material.

Pierre Baumann has recently got honoraries from AstraZeneca for CME courses teaching, as from many other pharmaceutical companies marketing psychotropic drugs in Switzerland.

Contributors

G. Nikisch, P. Baumann, G. Wiedemann, B. Kießling, H. Weisser, A. Hertel, T. Yoshitake, J. Kehr and A.A. Mathé contributed to the study. G. Nikisch oversaw the conduct of the study, and participated as first author. He executed the various procedures and wrote the first draft of the manuscript. B. Kießling, G. Wiedemann, A. Hertel and P. Baumann supervised the study planning, development and execution and contributed to the preparation of the manuscript. H. Weisser took part in the preparation of the samples for the *in vivo* study. A.A. Mathé and J. Kehr laboratories developed the assay techniques for monoamine metabolites, and A.A. Mathé and T. Yoshitake carried out the laboratory work. A.A. Mathé and J. Kehr also made significant contribution to writing up the manuscript.

All authors contributed to and have approved the final manuscript.

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Our funding sources had no involvement in the project or in the preparation of the project. In particular, they had no role in the interpretation of data; in the writing of the report and in the decision to submit the paper for publication.

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