Expression Changes of Serotonin Receptor Gene Subtype 5HT$_{3a}$ in Peripheral Blood Mononuclear Cells from Schizophrenic Patients Treated with Haloperidol and Olanzapine

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ABSTRACT

Serotonin receptors are involved in pathophysiology of schizophrenia and may mediate other neurotransmitter effects.

We investigated serotonin receptors gene expression in peripheral blood mononuclear cells (PBMC) of naïve schizophrenic patients, before and after treatment. Also serotonin receptor gene expression was compared in two treatment groups including Haloperidol and Olanzapine. The PBMC was separated from whole blood by Ficoll-hypaque. The total cellular RNA was extracted and the cDNA was synthesized. This process was followed by real-time PCR using primer pairs specific for 5HT$_{3a}$ serotonin receptor mRNA and beta-actin as internal control.

The results showed the presence of subtype of serotonin receptor in lymphocytes. Serotonin gene expression showed significant changes in Olanzapine treatment group which correlated with Clinical Global Impression (CGI) score improvement.

In conclusion, the present study has shown that human PBMC express serotonin receptors 5HT$_{3a}$. Moreover, clinical symptom improvement of Olanzapin may be demonstrated by a change in serotonin receptor gene expression.

Key words: Haloperidol; Olanzapin; Schizophrenia; Serotonin receptor; 5HT$_{3a}$

INTRODUCTION

Serotonin receptor subtype 5HT$_{3a}$ is an ion channel that is involved in etiological pathways of several neurological diseases.¹ The role of serotonin receptors in schizophrenia was described many years ago.²,³ Serotonin receptors are involved in pathology of...
Schizophrenia may mediate other neurotransmitter effects. Thus some studies reported its role in a number of schizophrenia symptoms.\textsuperscript{4,5} In spite of many studies that investigated on schizophrenia but there are not any study regarding the effects of 5HT\textsubscript{3a} in schizophrenia.\textsuperscript{6} This receptor is a member of the Cys-loop of ligand-gated ion channels, which also includes nicotinic acetylcholine (nACh), glycine, and GABAA receptors.\textsuperscript{1} Several antagonist drugs of serotonin–dopamine receptors like Olanzapine have been used for management of psychiatric disorders.\textsuperscript{7} The molecular structure of this drug has similar homology with clozapine and selectively can bind to several serotonin receptors subtypes 5HT\textsubscript{2a}, 5HT\textsubscript{2c}, 5HT\textsubscript{3}, 5HT\textsubscript{6} and dopamine receptors (D1-D4).\textsuperscript{8} Some reports showed beneficial treatment effects of Haloperidol in schizophrenia. Other studies indicated better benefits of Olanzapine in compared to Haloperidol in treatment of negative symptom and complications.\textsuperscript{7}

In spite of all pharmacologically treatments Schizophrenia, the proportion of treatment-resistant schizophrenia has been estimated as 20-40% in the schizophrenic patients, and this unfortunate situation in the clinical psychiatric field still remains unchanged even after the introduction of several atypical antipsychotic agents.\textsuperscript{9}

Blood products and peripheral blood mononuclear cells (BPMC) are readily accessible and may reflect molecular processes in the central nervous system of schizophrenic patients,\textsuperscript{10,11} although the validity of peripheral markers is still under debate. Substances in serum and PBMC that have previously been studied in schizophrenia include those related to the therapy, receptors for major neurotransmitters and G-protein subunits which regulate intracellular signal transduction.\textsuperscript{11,12}

This study was conducted to examine whether human PBMC could be useful in the study of the mechanism of antipsychotic treatment in schizophrenia patients. Based on this model we tried to compare effects of Haloperidol and Olanzapine on schizophrenia symptoms improvements and also to determine changes of serotonin receptors gene expression.

**PATIENTS AND METHODS**

Thirty schizophrenia patients (age 20-35 years) drug naive schizophrenic patients took part in this study. Diagnosis of these patients was based on structured clinical interview for DSM-IV (SCID). Exclusion criteria were psychiatric and neurological disorders and any chronic diseases like cardiovascular and endocrinological diseases. Also participant current inflammatory or infectious diseases were excluded from the study.

We tried to compare effects of Haloperidol and Olanzapine on schizophrenia symptoms improvements and according to these medications two treatments groups were defined. After 4 weeks of treatments changes of serotonin receptors gene expression in these groups were determined.

All measurements were performed before and after medications. A reduction of at least one category on the Clinical Global Impression (CGI) scale was considered as treatment response.

Written informed consent was obtained from each individual. Peripheral blood samples (4ml) were obtained from the cubital vein and collected in cell preparation tubes containing EDTA. Peripheral blood mononuclear cells were separated according to the methods previously described.\textsuperscript{13} After cells separation mRNA was extracted by RNA blood minikit (Roach, Germany).

Total RNA of 1500 ng was reverse-transcribed into first-strand cDNA using random hexamers and 2.5 units of multiscribe (recombinant Maloney murine leukemia virus).\textsuperscript{14} Primer for 5HT\textsubscript{3a} and housekeeping gene β-actin were designed using primer express software to exclude amplification of genomic DNA and pseudo genes.

cDNA of 75 ng was used for PCR amplification in a final volume of 25 μl with 1 unit of Taq DNA polymerase (Roach, Germany). PCR was carried out in a Real-time-PCR (Roach, Germany) with a Cyber green fluorogenic nucleotide to monitor cDNA amplification by the increase in Fluorescence intensity. Figure1 shows melting curve of serotonin receptor gene expression. PCR product of serotonin receptor (5HT\textsubscript{3a}) was sequenced by DNA sequencer ABI 3700 capillary system (Applied Biosystem, USA) to confirm amplicon sequence.

**RESULTS**

In this study we examined 5HT\textsubscript{3a}, serotonin receptor gene expression changes in the PBMC of Schizophrenic patients during 4 weeks of two treatments.
Expression Changes of Serotonin Receptor in Schizophrenia

Table 1. Gene expression of serotonin receptor (5HT\textsubscript{3a}) in two treatment groups

<table>
<thead>
<tr>
<th>Drug</th>
<th>5HT3a gene expression*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline*</td>
<td>After treatment*</td>
</tr>
<tr>
<td>Haloperidol**</td>
<td>0.0046 ± 0.0031</td>
<td>0.0054 ± 0.004</td>
</tr>
<tr>
<td>Olanzapine**</td>
<td>0.0035 ± 0.002</td>
<td>0.0025 ± 0.001</td>
</tr>
</tbody>
</table>

* Gene expression score of serotonin receptor was calculated as target gene concentration divided by beta actin concentration.
** Antipsychotic therapy for 4 weeks

Blood samples were taken and clinical state was assessed at baseline, and after 4 weeks of treatment, so that each patient served as his own control.

We focused on all subtypes of serotonin receptor 5HT\textsubscript{3a}. The experiment was performed using PBMC. Expression of the serotonin receptor gene segments was studied by analyzing total RNA extracted from the samples. In order to detect serotonin gene receptor expression on RNA level, Real time PCR was performed.

Our results presented here provide direct evidence that human PBMC express serotonin like receptors belonging to 5HT\textsubscript{3a} receptor in PBMC of schizophrenic patients. The taqman assays were tested by blasting against the entire human genome (NCBI-National Center for Biotechnology Information 2009) to exclude sequencing at unwarranted sites.\textsuperscript{15} The specificities of the obtained PCR products for the respective serotonin receptors fragments were confirmed by capillary sequenced analysis ABI 3700 machine.

In treatment follow up, significant changes were observed with mean CGI score (P=0.01). No significant differences were found in the CGI scales between the Olanzapine and Haloperidol treated sub-groups of patients. Serotonin receptor gene expression is shown. Changes of serotonin receptor gene according to kind of treatments are shown (Figure 2).

No statistically significant differences were found in the serotonin receptor gene expression before treatment between patients assigned to Olanzapine and Haloperidol groups. After treatment, serotonin receptor gene expression in Olanzapine treated patients was significantly decreased (P=0.01) but in Haloperidol group no significant gene expression changes were detected.

![Figure 1. Melting curve of serotonin receptor gene expression (5HT\textsubscript{3a})](image-url)
DISCUSSION

We found consistent gene expression changes in PBMC of schizophrenic patients undergoing two treatment protocols. This supports our suggestion that PBMC may be useful in investigating the mechanism of action of these drugs in clinical settings and it is in accordance with other researchers. There is a debate in relation to PBMC gene expression profile and similar changes in central nervous system. Related studies showed that serotonin receptors can be synthesized and can function within the central nervous system, as well as in lymphocytes. Also other studies confirmed the association between serotonin receptors expressions in central nervous system and peripheral lymphocytes.

In this study relationship between PBMC gene expression changes and clinical improvement was determined. Previous controlled studies demonstrated this relationship especially in improvement in primary negative symptoms.

Olanzapine, an atypical antipsychotic, has a broad receptor binding profile, which may account for its pharmacological effects in schizophrenia. Also 5HT3 serotonin receptor genes may be specific with kind of treatments that similar results reported in other studies. In vitro receptor binding studies related to Olanzapine showed a high affinity for all serotonin receptor which confirm our findings related to serotonin receptor gene expression in patients treated with Olanzapine. Also other studies demonstrated serotonin receptor mRNA expression in brain by treatment with Olanzapine.

In experimental study, it has been demonstrated that Olanzapine may effect serotonin receptor gene expression through the transduction mediator like Janus kinase 2 (Jak2) and transcription factors like Stat3. Our findings show no significant changes in serotonin receptor gene expression. Other studies verify Haloperidol effects by regulation of dopamine receptor mRNA expression in the brain. It seems that these two medications effects on schizophrenia symptom improvement by different mechanisms and main effect of Olanzapin may demonstrate by changing serotonin receptor gene expression.

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