Original Paper

Neuropsychobiology

Neuropsychobiology 2015;71:120–124
DOI: 10.1159/000370077

Received: July 15, 2014
Accepted after revision: November 11, 2014
Published online: April 9, 2015

Effect of Clozapine and Other Antipsychotics on the Level of Platelet-Associated Autoantibodies in Children with Schizophrenia: A Longitudinal Follow-Up Study

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Key Words
Schizophrenia · Childhood-onset schizophrenia · Biomarker · Platelet-associated autoantibodies · Immune system

Abstract
Background: Previous studies have demonstrated significantly higher blood titers of platelet-associated autoantibodies (PAA) in adult schizophrenia patients compared to normal healthy subjects. In addition, young adult schizophrenia patients at their early stages of the disorder displayed higher PAA titers than older patients with longer duration of the disorder. ***Aim:*** To assess longitudinally the blood titers of PAA in inpatients with childhood-onset schizophrenia at admission, after short- and long-term follow-up, and the correlation of these titers with the response to clozapine and other antipsychotic treatments. ***Methods:*** Thirty children, age range of 6–12 (mean ± SD: 9.6 ± 1.5 years), with DSM-IV TR schizophrenia in active psychotic state were assessed 3 times: at baseline, after short-term (8–17 weeks; n = 26) and after long-term follow-up (33–170 weeks; n = 19). The blood titers of PAA were analyzed using ELISA and expressed by a linear optical density (OD) scale. A test recording >1.4 OD units was predefined as the positive cutoff value. ***Results:*** On long-term follow-up, 9 out of the 17 children who were PAA-positive at baseline became PAA-negative: 7 already after 2 months of clozapine treatment and 2 following 3 years of risperidone treatment. Eight children remained PAA-positive during the entire study period. There was no significant correlation between the clinical improvement (as assessed by change in the Positive and Negative Syndrome Scale score) and the alteration in PAA levels (n = 19, r = –0.4, p = 0.088). ***Conclusions:*** High rates of positive PAA in COS patients may indicate an active autoimmune process in early-onset schizophrenia. It is concluded that PAA may serve as a biomarker for the diagnosis of COS, but does not predict the response to treatment. A transition to a PAA-negative status does not indicate an improvement in psychosis.

Introduction

Distinct features of immune dysfunctions associated with autoimmune processes have been implicated in some schizophrenia patients [1]. Most of the reported immune dysregulations in schizophrenia are associated with central pathophysiological mechanisms as well as with clinical manifestations of the disorder [1–3]. It has been

0302–282X/15/0712–0120$39.50/0

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suggested that in a distinct group of patients, psychotic symptoms may originate from an autoimmune reaction against platelets [4–6]. Accordingly, platelet-associated autoantibodies (PAA), which can cross-react with brain tissue, penetrate the blood-brain barrier (BBB) following an inflammatory or a traumatic insult [7], which may lead to the emergence of psychotic symptoms [5, 8]. Previous studies have demonstrated significantly higher blood titers of PAA in adult schizophrenia patients compared to normal healthy subjects [4–6]. In addition, young schizophrenia patients at their early stages of the disorder displayed higher PAA titers than older patients with long duration of the disorder [9–11].

Childhood-onset schizophrenia (COS) is a rare and severe form of schizophrenia characterized by onset of psychotic symptoms before age 12 [12, 13]. The frequency of COS is reported to be less than 1 case in 10,000–30,000 children. There is an extreme paucity of immunological studies in COS [14, 15]. High blood titers of PAA in this group of patients have been investigated and reported in our previous study [16].

The aim of the present longitudinal study was to determine the blood levels of PAA in children with COS: first when they are hospitalized in an acute psychotic state and later following short- and long-term intervals. By using this follow-up approach we wished to evaluate the relationship between the clinical progression of the disorder and the PAA titers.

We hypothesized that transition of a patient to negative PAA status will be associated with improvement in psychotic symptoms.

Methods

Subjects

The study participants were children with schizophrenia, age range 6–12 years, who were admitted to the Ness-Ziona Mental Health Center while in acute psychotic state. Inclusion criteria required that the participating patients meet the DSM-IV TR diagnostic criteria of schizophrenia, following a structured interview according to the guidelines of the Structured Clinical Interview for Axis-I DSM-IV TR Disorders – Patient Version (SCID I/P) [17] and exhibit a minimal score of 60 on the Positive and Negative Syndrome Scale (PANSS) [18]. The level of functioning at admission was evaluated by the Clinical Global Impression Scale – Severity (CGI-S) score [19], ranging from 4 (moderately ill) to 7 (extremely ill).

Patients with past or current medical or neurological illness, or current major routine laboratory abnormalities, were excluded from the study. In addition, treatment-resistant patients (patients who did not respond to at least two antipsychotics administered in adequate doses for at least 6 weeks) treated with clozapine at admission were also excluded from the study. All patients underwent physical examination prior to the beginning of the study. Data regarding physical diseases were collected from the medical records and interviews of parents. The study was approved by the Beer Yaakov – Ness-Ziona Institutional Review Board and both parents signed a written informed consent for participation of their children in the study. Informed consent was obtained from the children themselves as well.

Laboratory Procedure

A procedure outlined in our previous publications [9–11] was used. Ten milliliters of venous blood was collected from each of the participants. Serum samples were separated within 12 h and then stored at –20°C until assayed for the PAA. Titers of PAA were evaluated by a previously described ELISA procedure [9] which was optimized to a highly reproducible test. In this test, an immobilized dimer of a peptide epitope from a specific platelet antigen [9] provided the binder of circulating PAA, followed by an enzymatic color release proportional to the level of bound PAA, expressed in units of optical density (OD). The color intensity scale was based on preevaluated empirical cutoff OD of 1.4 units [9], below and above which the test was determined as negative or positive, respectively.

Study Design

Baseline PAA titers of 29 COS patients were described previously [16]. In the current study we included 1 additional child. Titers were assessed after a short-term period (8–17 weeks), as well as after a long-term period (33–170 weeks). In total, we collected data for the three time points (baseline, short-term and long-term follow-up) on 19 children with COS.

Statistical Analysis

Baseline blood titers of PAA of the COS patients were compared with PAA titers of the same subjects after a short-term and a long-term period, using a two-tailed paired Student's t test. Correlation between alterations in PAA titers and PANSS scores was evaluated using Pearson's correlation test. Categorical data were analyzed using Fisher's exact test. All values are expressed as mean ± SD.

Results

The clinical description of the original study population was previously described [16]. As mentioned above, 1 additional child was included in the present study. In the current follow-up, we included participants that were monitored for a period of at least 30 weeks. We analyzed the impact of antipsychotic treatment on PAA status at three time points: baseline, short-term and long-term follow-up, as described below. There were no cases of agranulocytosis in either the short-term or the long-term follow-up periods.

Baseline versus Short-Term Follow-Up (n = 26)

At the short-term follow-up, we had data only for 25 out of the 29 children who originally participated in the study [16], plus data on the additional child. The aver-

Table 1. Alterations in PANSS score and PAA levels during short- and long-term follow-up periods (n = 26)

Parameter	Baseline	Short-term follow-up	Short-term percent of decrease (of baseline levels)	Long-term follow-up	Long-term percent of decrease (of baseline levels)
Nonclozapine treatment[1]					
PANSS score	111.3±21	85.9±17.13	23.4	80.7±11.9	27.0
CGI-S score	5.5±0.8	4.1±0.8	25.5	4.3±0.8	22.0
OD (PAA levels)	1.85±0.5	1.71±0.6	4.0	1.75±0.5	3.0
Clozapine treatment (n = 8)					
PANSS score	113.0±10.3	112.0±14.7	0.9	103±14.9	8.5
CGI-S score	5.8±0.5	5.8±0.8	0	5±0.8	14.0
OD (PAA levels)	1.98±0.4	1.25±0.6	37.0	0.97±0.3	50.0

[1] Short-term follow-up: n = 18; long-term follow-up: n = 11.

Table 2. Alterations in PAA status (positive vs. negative) and PANSS score following short- and long-term follow-up

	PAA-positive	PAA-negative
Nonclozapine treatment (n = 11)		
Baseline	10	1
Short-term follow-up	10	1
Short-term decrease in PANSS, % of baseline	23.0	7.0
Long-term follow-up	8	3
Long-term decrease in PANSS, % of baseline	27.0	19.0
Clozapine treatment (n = 8)		
Baseline	7	1
Short-term follow-up	0	8
Short-term decrease in PANSS, % of baseline	–	0
Long-term follow-up	0	8
Long-term decrease in PANSS, % of baseline	–	8.5

age duration of the follow-up was 13.5 ± 3.0 weeks, at a range of 8–17 weeks. One child was monitored only after 1 year and was included only in the long-term follow-up.

The actual alterations in PANSS scores and PAA titers are described in table 1.

As shown, a decrease of 23.4 and 25.5% was observed in the total PANSS score and CGI-S score, respectively, following treatment with agents other than clozapine. In contrast, there were no decreases in PANSS or CGI-S scores in patients who were treated with clozapine for the same period of time. Clozapine treatment was ineffective in reducing the psychotic symptoms (0.9% decrease in PANSS score, 0% decrease in CGI-S score), however, the treatment was associated with a marked decrease in PAA titers (37%), which was not observed in patients treated with nonclozapine drugs (a decrease of 4% in PAA titers).

We then divided the patients into two status groups: PAA-positive and PAA-negative (using the cutoff point of OD ≥1.4). The clinical and immunological data of these two groups are described in table 2. As shown, in the short-term follow-up group, at admission, 4 children were PAA-negative (OD <1.4), and 22 children were PAA-positive. Following the short-term follow-up, 3 PAA-negative children, who were treated with nonclozapine antipsychotics, and 1 child who was switched to clozapine during this time period, remained PAA-negative. All children who were PAA-positive and were treated with nonclozapine antipsychotics remained PAA-positive. Seven children who were switched to clozapine became PAA-negative already after 2 months of treatment with this agent.

Baseline versus Long-Term Follow-Up (n = 19)

Only 19 children agreed to participate in the long-term follow-up (73.5 ± 41.0 weeks, range 33–170 weeks). Eight of them were treated with clozapine and 11 with other antipsychotics.

A further mild decrease was observed in the nonclozapine-treated patients at the end point of the long-term treatment (27.0% decrease in PANSS score compared to 23.4% decrease in the short-term follow-up; 22.0% decrease in CGI-S score compared to 25.5% decrease in the short-term follow-up). A moderate decrease in both PANSS and the CGI-S scores (8.5 and 14.0%, respectively) was obtained following the long-term clozapine treatment, in contrast to the nonresponsiveness in the short-term period. No further decrease in PAA levels was observed in the patients treated with nonclozapine agents while an additional decrease in the PAA levels was observed in the clozapine-treated group (50.0 vs. 37.0%).

Platelet-Associated Autoantibodies. In this long-term follow-up group (n = 19), 2 children were PAA-negative and 17 were PAA-positive at the beginning of the study (table 2). No significant difference in the levels of PAA between the clozapine treatment and the nonclozapine treatment groups at the beginning and follow-up was observed (p = 1.00, n.s.). After the long-term follow-up, 9 of these children became PAA-negative: 2 following about 3 years of risperidone treatment and 7 following merely 2 months of clozapine treatment. Eight children who were PAA-positive at baseline remained PAA-positive following long-term treatment with nonclozapine agents. It should be noted that the 7 children who became PAA-negative after the short-term treatment with clozapine remained PAA-negative after the long-term treatment with this agent. A significantly higher rate of transition to PAA-negative status was observed in the clozapine-treated patients in comparison to the nonclozapine-treated patients following the long-term treatment (7/7 vs. 2/9, respectively; p = 0.0034, OR = 44.29, 95% CI 1.84–928.21).

Relationship between Changes in PAA Levels and PANSS Scores Following Long-Term Treatment

In the total study population, no significant correlation was found between the clinical improvement achieved following long-term treatment (as demonstrated by decline in the PANSS score) and the change in actual PAA levels, expressed in OD values (n = 19, r = –0.4, p = 0.088).

In the nonclozapine-treated children who exhibited clinical response to treatment, there was no significant correlation between the decrease in PANSS score and the change in PAA levels (n = 11, r = –0.27, p = 0.43). The same was true for the clozapine-treated children: no significant correlation was observed between the decline in PANSS score and the decline in PAA levels following long-term treatment (n = 8, r = 0.45, p = 0.26). Thus, it appears that there was no relationship between the changes in PAA levels and/or status and the clinical improvement in the severity of psychosis.

Discussion

It has been previously reported that the serum of schizophrenia patients is enriched with PAA [4, 6, 10, 11] and an inverse correlation has been detected between the duration of disease and the level of PAA titers [10, 11, 16]. The main objective of the present study was to assess the impact of clozapine and nonclozapine antipsychotics on PAA levels in COS patients and to evaluate a possible relationship between clinical improvement and alterations in PAA levels.

Most of the COS patients were PAA-positive at baseline (17/19). The presence of high PAA titers may have indicated an active autoimmune process associated with the pathophysiology of COS, and it was suggested that a PAA-positive status may serve as an immune biomarker of COS [16]. We have previously observed [5, 8, 10] that the PAA levels in adolescent and adult schizophrenia patients were not influenced by treatment with any of the antipsychotic medications, except clozapine. Similarly, in the present study it was clearly demonstrated that there was no correlation between changes in PAA status and the clinical improvement following antipsychotic treatment. With respect to clozapine, the major finding of this study was that clozapine treatment is associated with conversion of PAA status from PAA-positive to PAA-negative in both short-term and long-term treatments. This observation may reflect a possible immunomodulatory activity of clozapine, which is independent of its antipsychotic activity, at least in relation to PAA. Immunomodulatory effects of clozapine were reported previously regarding in vivo cytokine synthesis and release [20]. The immunosuppressive effect observed in our study may be related to a nonspecific cytotoxic effect of this agent [20, 21]. However, none of our patients developed agranulocytosis during the clozapine treatment, thus the relationship between the suppression of immunocompetent cells and the reduction in PAA levels remains speculative. Nevertheless, it is obvious that there is no relationship between the immunomodulatory effect of clozapine observed in our study and the clinical improvement. From

a clinical point of view, the effectiveness of clozapine in the COS patients who, by definition, did not respond to other antipsychotics, was very mild. However, the small sample size of this group does not allow the generalization of this observation. It is of note that at baseline, the clozapine and nonclozapine treatment groups did not differ in PAA levels or the rate of PAA-positive status, thus it appears that PAA is not a marker for nonresponsiveness to antipsychotic treatment.

In conclusion, COS is characterized by a high rate of PAA-positive status, and clozapine, but not nonclozapine treatment, is associated with conversion to PAA-negative status, which does not correlate with a parallel clinical improvement. Moreover, a relatively good response was monitored during the two follow-up periods in the nonclozapine-treated patients. However, the achieved clinical improvement was not accompanied by a corresponding change in PAA status.

Nonetheless, the notion that autoantibodies against a dimerized platelet epitope that cross-react with sequestered antigen in the brain may have pathogenic consequences when they cross a disrupted BBB and gain access to the brain cannot be disregarded. It is therefore possible that an inflammatory process at an early age can lead to disruption of the BBB and penetrance of the autoantibodies into the brain, resulting in neuropsychiatric disorders such as autism [22]. Unfortunately, we do not have data showing that PAA-positive children were more likely to have an illness associated with BBB disruption prior to the onset of COS, as compared to the PAA-negative group.

To our knowledge, at present there are no animal models that have explored the behavioral effects of PAA after experimental disruption of the BBB.

Later, at the chronic stage, the association between the severity of the disease and the PAA status is lost, and the PAA levels do not reflect the symptomatic improvement. The complex relationship between the presence of PAA and the pathophysiology and pharmacotherapy of COS merits further, large-scale studies.

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