Hyperprolactinemia in Patients on Antipsychotic Drugs Causes ADP-Stimulated Platelet Activation That Might Explain the Increased Risk for Venous Thromboembolism: Pilot Study

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Abstract: Recently, an increased risk of venous thromboembolism in patients on antipsychotic drugs has been reported, but the molecular etiology is still unknown. Most antipsychotic drugs act as dopamine antagonists, and some of them cause hyperprolactinemia. Hyperprolactinemia has recently been found to cause increased platelet activation via potentiating ADP effects on human platelets.

We assessed prolactin values as well as ADP-stimulated and thrombin receptor activator 6-stimulated expression of the platelet activation marker P-selectin in 20 consecutive patients under therapy with antipsychotic drugs.

We detected a significant correlation between prolactin values and ADP-stimulated P-selectin expression on platelets in patients on antipsychotic drugs, revealing a significant higher platelet stimulation in hyperprolactinemic patients on antipsychotic drugs than in normoprolactinemic controls.

Therefore, hyperprolactinemia might be the yet unknown acquired risk factor in patients on antipsychotic drugs explaining the increased risk for venous thromboembolism in these patients.

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Recently, Zornberg and Jick[1] demonstrated in a population-based, nested case-control analysis that the use of antipsychotic drugs increases the risk of idiopathic venous thromboembolism (VTE). This finding is clinically important because antipsychotic drugs are widely used in psychiatry and general medicine. The increased risk for VTE seemed to be an effect of current exposure to antipsychotic drugs. There was no evidence for modification of this effect by age, sex, and concomitant antidepressant or estrogen use. Until now, several mechanisms have been postulated to explain this effect. At first, it has been hypothesized that venous stasis is increased by the sedation in patients on antipsychotic drugs and might contribute to the increased risk for VTE.[1] One further possible explanation is the presence of anticardiolipin antibodies, which is associated with an increased risk of VTE and is raised in some patients on chlorpromazine.[2] A third possible mechanism is the enhanced platelet aggregation by antipsychotic drugs, as it was suggested by former studies demonstrating that changes of 5-hydroxytryptamine are associated with platelet aggregation in patients on fluphenazine, but this phenomenon has not been explained in detail.[3] However, all these postulated mechanisms have not been replicated.

Beyond the well-known plasmatic factors, the pathogenesis of thrombosis involves complex platelet-leukocyte interaction whereby details are not fully elucidated.[4] P-selectin mediates rolling of platelets and leukocytes on activated endothelial cells. Recent data indicate that the P-selectin interaction with a ligand stabilizes initial GP IIb/IIIa-fibrinogen interactions, thus allowing the formation of large stable platelet aggregates.[5] These studies suggest that the activation of platelets is an initial step in the development of venous thrombosis. Until now, the significance of activated platelets for the development of VTE is not systematically investigated, whereas the influence of platelet activation on arterial thrombosis is undisputed.

Recently, we have investigated platelet function abnormalities in hyperprolactinemic states, like pregnancy and in patients with pituitary tumors, demonstrating a

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significant correlation between prolactin (PRL) values and enhanced ADP stimulation of platelets.[6] Hyperprolactinemic pregnant women or hyperprolactinemic patients with pituitary tumors revealed significantly higher ADP stimulation of platelets than healthy controls or normoprolactinemic patients with pituitary tumors. These results were reconciled by an increased in vitro stimulation and aggregation of platelets using human PRL.[6] Moreover, in a second study, we determined plasma PRL levels in healthy subjects and patients with VTE, demonstrating that patients with VTE and no other congenital thrombophilic risk factors had significantly increased plasma PRL levels. Moreover, patients with prolactinomas demonstrated a higher incidence of VTE than the general population.[7] These findings indicate that hyperprolactinemia may be an important novel risk factor for VTE, and its thrombogenic effect is mediated through enhanced platelet reactivity. It is well known that antipsychotic drugs act as dopamine antagonists and that most of them cause hyperprolactinemia. Therefore, we hypothesized that elevated levels of PRL in patients on antipsychotic drugs cause increased platelet activation and aggregation that might be the reason for the increased risk of VTE in these patients.

SUBJECTS AND METHODS

We assessed PRL values as well as ADP-stimulated and thrombin receptor activator 6 (TRAP-6)-stimulated expression of the platelet activation marker P-selectin in patients under therapy with antipsychotic drugs, as previously described.[6] Full verbal and written consent was obtained from all participants after the procedures had been fully explained. Twenty consecutive patients (mean age 41 ± 10 years; 14 females, 6 males) on antipsychotic drugs without a history of VTE and further medication were investigated. Clinical and laboratory data as well as antipsychotic medication are shown in Table 1. One hundred healthy people (mean age 30 ± 18 years; 46 females, 54 males) without any medical therapy served as controls for ADP stimulation of platelets. All 100 controls showed normal values of PRL.

Determination of Serum Prolactin

The serum PRL values of healthy controls and patients on antipsychotic drugs were determined with the Axsym Prolactin Assay (Abbott, USA). This assay was performed according to the manufacturer's instructions. Serum PRL values were considered as normal if they were <580 mU/L (24.2 ng/mL) in females or <450 mU/L (18.77 ng/mL) in males, as previously described.[6]

Flow Cytometric Platelet Analysis

The determination of ADP-stimulated, TRAP-6-stimulated, and nonstimulated P-selectin expression of platelets using a flow cytometric method was performed as previously described.[6–8] Citrated whole blood from healthy donors, pregnant women, and patients suffering from pituitary tumors was diluted in Hank balanced salt solution (Sigma, Deisenhofen, Germany) containing 1-mg/mL bovine serum albumin (Sigma). Platelet count was adjusted to ~20,000 platelets per microliter. Aliquots of the platelet suspension were activated for 10 minutes at 37°C with 5-μM ADP (final concentration; Sigma) and 10-μM TRAP-6 (final concentration; Bachem, Bubendorf, Switzerland). Native nonactivated whole blood from the same sample served as control. The aliquots were incubated at 37°C with saturating concentrations of a murine PE-labeled anti-GP IIb/IIIa monoclonal antibody (clone P2, CD41; Beckman Coulter, Krefeld, Germany) and a murine fluorescein isothiocyanate-labeled anti-GMP 140 monoclonal antibody (clone CLB-Thromb/6, CD62P; Beckman Coulter). Platelet activation and staining were stopped after 5 minutes of incubation with 2 mL of 4°C Hank balanced salt solution buffer. A Beckman Coulter EPICS XL flow cytometer equipped with standard filters for fluorescein isothiocyanate and PE fluorescence analysis and an XL System II software R 2.0 (Beckman Coulter, Fullerton, CA, USA) was used for measurements. Platelets were gated in the forward scatter versus fluorescence 2 (CD41-PE) dot plot based on the characteristic forward scatter signal and the high CD41-PE signal. To exclude all CD41-negative events, the discriminator was set in fluorescence 2. In the list mode, 10,000 platelets were acquired at a flow rate of <500 particles per second and were subsequently analyzed. All assays were performed in at least 2 separate experiments. The reference range for ADP-stimulated P-selectin expression of platelets was determined using blood from 100 healthy blood donors (mean fluorescence intensity in arbitrary fluorescence units: 4.88 ± 1.35 [mean ± 2 SD]). Results above the 2 SD limit of the mean value of the 100 control subjects (6.23) were considered positive for increased ADP stimulation, as previously described.[6–8] Flow cytometric platelet analysis was performed in duplicate in at least 2 separate experiments.

Platelet Aggregometry

Freshly drawn venous blood was collected into 0.1-vol 3.8% trisodium citrate. Platelet-rich plasma was obtained after centrifugation of the citrated whole blood (150*g*, 15 minutes). The aggregation studies were performed using a PAP-4C aggregometer (Bio/Data, Horsham, PA, USA) in 250-μL platelet-rich plasma adjusted to 200,000 platelets per microliter with platelet-poor plasma. Platelet-rich plasma was preincubated for 5 minutes at 37°C with different concentrations of plasma human PRL and was activated using 5-μM ADP. Optical density of platelet-poor plasma was set to 100% and that of platelet-rich plasma to 0% before addition of activators, as previously reported.[6–8] Platelet

aggregometry was performed in duplicate in at least 2 separate experiments.

Statistical Analysis

In previous studies, we detected a linear correlation between PRL values and ADP stimulation of platelets in pregnant women and in patients with pituitary tumors.[6] Therefore, we calculated the Pearson correlation coefficient in the present study. The Mann-Whitney *U* test was used to compare the ADP stimulation of platelets among healthy controls, normoprolactinemic patients, or hyperprolactinemic patients on antipsychotic drugs, as previously reported.[6] For multiple comparisons, Bonferroni correction was used. *P* values of <0.05 were considered as statistically significant. Data were analyzed with SPSS for Windows (realized 9.0.1).

RESULTS

Six of the 20 patients showed normoprolactinemia (3 in the upper tertile and 3 in the middle tertile of the upper range of normal values) and 4 patients with only a slight increase of PRL values (PRL < 1000 mU/L), whereas 10 patients

TABLE 1. Clinical and Laboratory Data of 20 Consecutive Patients on Antipsychotic Drugs

No.	Sex	Age, (years)	Body Mass Index, kg/m^2	Diagnosis	Antipsychotic Drugs Per Day	Platelet Stimulation Index	PRL, mU/L
1	M	35	29	Depressive episodes	200-mg clozapine	5, 3	129
2	F	61	29	Schizophrenia	10-mg olanzapine	6, 3	268
3	F	56	23	Schizophrenia	10-mg olanzapine	6, 8	294
4	F	53	37	Depressive episodes	4-mg haloperidol + 75-mg melperon	5, 5	368
5	M	53	22	Schizophrenia	1-mg haloperidol	5, 1	408
6	F	60	21	Schizophrenia	50-mg melperon	5, 7	453
7	M	56	31	Depressive episodes	100-mg quetiapin	5, 2	580
8a	F	67	25	Schizophrenia	5-mg haloperidol	4, 3	425
8b					5-mg haloperidol + 15-mg olanzapine	6, 6	653
9	F	67	22	Depressive episodes	20-mg olanzapine	5, 5	666
10	M	30	31	Schizophrenia	20-mg olanzapine	3	700
11	M	44	28	Schizophrenia	500-mg sulpiride	8, 7	1064
12	M	62	27	Delirious states	6-mg risperidone	4, 4	1092
13a	F	51	30	Depressive episodes	22.5-mg haloperidol + 300-mg levomepromazin	4, 5	1217
13b					22.5-mg haloperidol + 300-mg levomepromazin + 20-mg olanzapine	6, 15	1553
14	F	78	21	Delirious states	1.5-mg haloperidol + 75-mg melperon	8, 2	1479
15	F	51	30	Depressive episodes	100-mg levomepromazin + 20-mg flupentixol + 20-mg olanzapine	5, 2	1553
16	F	39	23	Depressive episodes	200-mg sulpiride + 37.5-mg melperon	7, 2	1976
17	F	50	26	Schizophrenia	6-mg risperidone + 20-mg olanzapine	8, 3	1985
18	F	52	22	Schizophrenia	20-mg olanzapine	5	2080
19	F	24	23	Depressive episodes	10-mg olanzapine	6, 9	2319
20a	F	39	15	Depressive episodes	400-mg sulpiride	7, 2	1976
20b					400-mg sulpiride + 27.5-mg melperon	9, 2	2499

This shows the clinical and laboratory data of all investigated patients. The PRL values and ADP-stimulated P-selectin expression (platelet stimulation index) are shown during therapy with antipsychotic drugs. One patient (patient 7) was investigated after the first dose of therapy. For 3 patients (patients 8, 13, and 20), we determined PRL values and ADP-stimulated P-selectin expression twice after adding further antipsychotic drugs. All other patients were investigated during stable therapy.

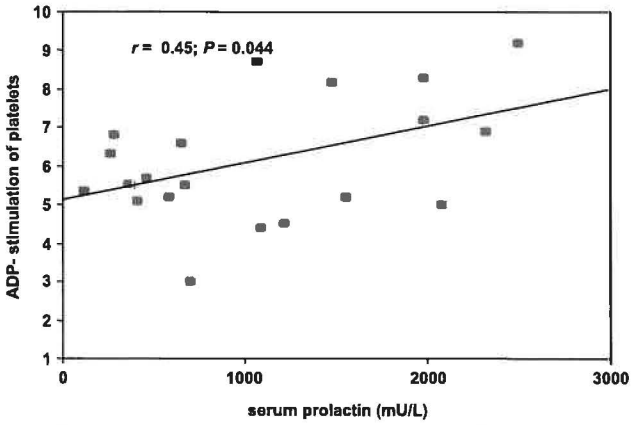

FIGURE 1. Correlation between ADP stimulation of platelets and serum PRL in 20 patients on antipsychotic drugs. The correlation between ADP stimulation of platelets presented as P-selectin expression and PRL serum values in 20 consecutive patients on antipsychotic drugs was analyzed and quantified with the Pearson correlation coefficient.

revealed hyperprolactinemic values (PRL > 1000 mU/L; Table 1). In all 20 patients, the PRL values were significantly correlated with the ADP-stimulated P-selectin expression of platelets (Pearson correlation coefficient $r = 0.45$, $P = 0.044$; Fig. 1). Furthermore, both groups of patients with highly elevated PRL values (PRL > 1000 mU/L) or only slight increased to normal of PRL values (PRL < 1000 mU/L) revealed significantly higher ADP-stimulated P-selectin expression than did the 100 healthy control persons (Mann-Whitney U test $P = 0.00016$ or 0.0047, respectively; Fig. 2). For 3 patients (patients 8, 13, and 20), we determined PRL values and ADP-stimulated P-selectin expression twice after adding further antipsychotic drugs (Table 1). In all patients, the change of therapy caused both increased PRL values and increased P-selectin expression on platelets already after a short period (days). Furthermore, increased P-selectin expression was reconciled by increased in vitro platelet aggregation in patient 8 (data not shown). In contrast to ADP-stimulated P-selectin expression, the TRAP-6 stimulation of platelets was not influenced by PRL values (data not shown).

DISCUSSION

Beyond the well-known plasmatic factors, the pathogenesis of thrombosis involves complex platelet-leukocyte interactions.[4,5] P-selectin mediates rolling of platelets and leukocytes on activated endothelial cells stabilization of initial GP IIb/IIIa-fibrinogen interactions, thus allowing the formation of large stable platelet aggregates.[4,5] Furthermore, in the Pulmonary Embolism Prevention Trial, it has been demonstrated that aspirin can reduce VTE by at least a third throughout a period of increased risk.[9] These data suggest that the activation of platelets is an important initial step in the development of venous thrombosis. To date, there is no sufficient explanation for the reported increased risk for VTE in patients on antipsychotic drugs.

In our recent study, we have demonstrated a correlation between PRL levels and platelet activation in pregnant women and patients with hyperprolactinemic pituitary tumors.[6] In a second study, we have demonstrated that patients with VTE without congenital or acquired risk factors had significantly higher PRL levels than those with congenital risk factors or healthy controls. Furthermore, patients with prolactinomas had a significantly higher incidence of idiopathic VTE than the general population.[7] These findings indicate that hyperprolactinemia may be an important novel risk factor for VTE and its thrombogenic effect is mediated through enhanced platelet reactivity.

To the best of our knowledge, our study is the first one in investigating PRL values and ADP-stimulated P-selectin expression on platelets in patients on antipsychotic drugs. In patients on antipsychotic drugs, the PRL values were correlated with the ADP-stimulated P-selectin expression of

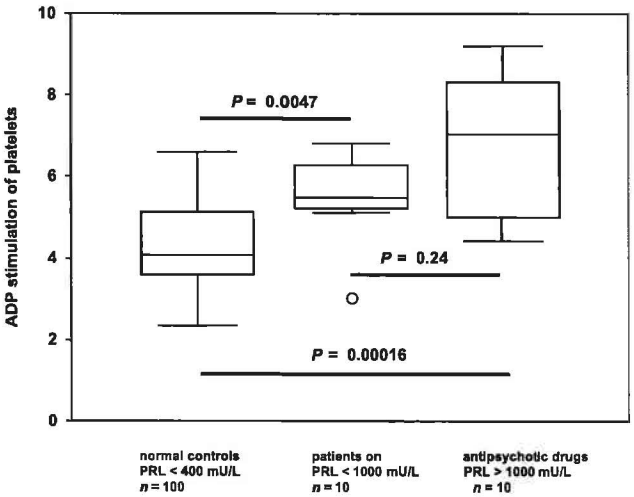

FIGURE 2. ADP stimulation of platelets in healthy controls, normoprolactinemic patients, or hyperprolactinemic patients on antipsychotic drugs. Six of the 20 patients showed normoprolactinemia (3 in the upper tertile and 3 in the middle tertile of the upper range of normal values) and 4 patients with only a slight increase of PRL values (PRL < 1000 mU/L), whereas 10 patients revealed hyperprolactinemic values (PRL > 1000 mU/L). Both groups of patients with highly elevated PRL values (PRL > 1000 mU/L) or only slight increased to normal of PRL values (PRL < 1000 mU/L) revealed significantly higher ADP-stimulated P-selectin expression than did the 100 healthy control persons (Mann Whitney U test $P = 0.00016$ or 0.0046, respectively). Box plots represent 25th and 75th percentiles. Error bars represent 10th and 90th percentiles. The dot is a single value below the 10th percentile. P values are given above and below the plots.

platelets in accordance to previous findings in other hyperprolactinemic states.[6] We are aware that the observed correlation between P-selectin expression on platelets and PRL is statistically weak ($r = 0.45$, $P = 0.044$), depending on the relatively small number of patients in our pilot study. However, in a previous study with 42 pregnant women and 22 patients with pituitary tumors, we observed a linear correlation between P-selectin expression and PRL with higher statistically significance ($r = 0.56$, $P = 0.0001$) but with almost the same correlation coefficient. This association between PRL and platelet P-selectin expression has been reconciled for many times in in vitro studies[6,7]

Our patients on antipsychotic drugs with highly elevated PRL values (PRL > 1000 mU/L) or only a slight increased to normal PRL values (PRL < 1000 mU/L) revealed significantly higher ADP-stimulated P-selectin expression than did the 100 healthy control persons. Of the 6 patients with normoprolactinemia, 3 had values in the upper tertile and 3 in the middle tertile of the upper range of our PRL assay. These results correspond to our previous in vitro data showing an increased dose-dependent platelet aggregation by PRL starting already in the upper reference range about 300 mU/L.[6] It has been demonstrated that atypical antipsychotic drugs, like olanzapine, clozapine, and ziprasidone, cause only minimal increases of PRL values in adults, which may be related to a higher 5-hydroxytryptamine$_{2A}$-D2 binding.[10] Therefore, both groups, typical and atypical antipsychotic drugs, should differ in their influence on platelet activation. Paradoxically, in our study, we had 2 patients on olanzapine (patients 18 and 19; Table 1) who revealed PRL levels > 2000 mU/L. Furthermore, both patients showed increased P-selectin expression on platelets, demonstrating the association of PRL increase and platelet activation in patients on atypical antipsychotic drugs. Therefore, for single patients, the predictability of PRL increases by antipsychotic drugs may not be fully explained by the type of drugs or their mechanism of action.

In our previous study, we had observed short-term changes of PRL and ADP-stimulated P-selectin expression during dopamine agonist therapy (days) or in TRH test (minutes), respectively.[6] Now, we can demonstrate such parallel changes of PRL values and ADP stimulation of platelets in patients on antipsychotic drugs (patients 8, 13, and 20; Table 1). In all patients, the change of antipsychotic therapy caused both increased PRL values and increased P-selectin expression on platelets already after a short period (days). Moreover, this increase of P-selectin expression could be reconciled by an increase of platelet aggregation in vitro for at least 1 patient (patient 6; Table 1).

To elucidate the molecular mechanisms for the PRL-stimulated platelet aggregation, we have investigated PRL receptor signaling during platelet activation with a focus on ADP-stimulated G protein-regulated signaling pathways. The short isoform of the PRL receptor was detected on platelets. The signaling through the PRL receptor, although not directly linked to Gq proteins, substitutes for Gq protein-regulated signaling pathways involved in platelet activation. We identified protein kinase C, a well-established signaling molecule in platelet activation, as a target molecule for PRL signaling pathways in human platelets.[7]

In conclusion, this study shows that hyperprolactinemia may be an additional yet unknown acquired risk factor for VTE in patients on antipsychotic drugs that might explain the increased risk for VTE in these patients in the absence of the known congenital risk factors. Therefore, a prospective study has been started to clarify the clinical relevance of hyperprolactinemia as a potential risk factor for VTE in patients on antipsychotic drugs.

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