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The effect of atypical antipsychotics on platelet aggregation

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ABSTRACT

Background: *There is a high prevalence of arterial thrombosis in schizophrenia. Several studies investigated the cause of this high risk among schizophrenic patients and variable finding reported. The aim of this study was to investigate whether second generation antipsychotics (risperidone, olanzapine and ziprasidone) exert antiplatelet action in the presence of different platelet agonists.*

Methods: *We performed an in vitro study of different antipsychotics (risperidone, olanzapine and ziprasidone) effect on platelet aggregation induced by different platelet agonists (ADP, collagen, serotonin and epinephrine) when added to blood of healthy volunteers using Multiplate® analyzer.*

Results: *Risperidone and ziprasidone but not olanzapine showed clinically significant inhibition of platelet aggregation induced by by serotonin, while only ziprasidone showed statistically significant inhibition on serotonin aggregation. All tested antipsychotics showed clinically (but not statistically) significant inhibition on platelet aggregation induced by epinephrine. On the other hand, no remarkable effect of antipsychotics on platelet aggregation induced by ADP or collagen was observed. Marked amplification reaction between serotonin and epinephrine was observed (AUC 66 U). When antipsychotics were added to serotonin-epinephrine combination, all of them produced AUC inhibition in a dose-dependent manner with highest potency for risperidone (IC50= 14.86 nM) and the lowest potency for olanzapine (IC50= 27.56 nM).*

Conclusion: *All tested antipsychotics showed clinically (but not statistically) significant inhibition effect on platelet aggregation induced by either serotonin or epinephrine. All antipsychotics studied inhibited platelet aggregation induced by a combination of serotonin and epinephrine in a dose-dependent manner.*

INTRODUCTION

Schizophrenia is a common form of psychotic disorders that affects more than one percent of the population [1] and it is one of the world's top causes of disability [2].

Antipsychotic medications have efficacy in the treatment of schizophrenia and other psychotic conditions where they show significant improvement in psychotic symptoms and quality of life in most patients [3].

Second generation antipsychotics (SGAs) are characterized by relatively potent antagonism of 5-HT_{2A} receptors with weaker antagonism of dopamine D2 receptors [3].

Serotonin 5-HT_{2A} receptor is also present on

platelets where it mediates platelet aggregation [4], thus, psychotropic drugs with high affinity and antagonism for this receptor subtype [5] are expected to have dual effect both on psychotic symptoms and platelet aggregation [6, 7].

Different platelet aggregation agonists bind specific platelet receptors and this lead to different responses in the hemostatic function of platelets [4, 8].

Platelet agonists are classified as strong such as thrombin or weak such as serotonin (5-HT) and epinephrine [4, 8].

There are 2 purinergic receptors to adenosine diphosphate (ADP) on platelets including P2Y1 and P2Y12. P2Y1 is responsible for platelet shape change and transient aggregation, P2Y12 has several roles in platelet that result in thrombus growth and stability [4, 8].

Serotonin binds 5-HT_{2A} receptors on platelets and amplifies the platelet response by stimulating shape change and recruiting more platelets to sites of injury [4].

In addition to its role in the initiation phase, collagen serves as a platelet agonist by binding to glycoprotein (GP) VI collagen receptors on the platelet surface and induces release of TXA2 and ADP [4, 8].

Although it is considered a weak agonist, epinephrine acts synergistically (by binding to α_{2A}-adrenergic receptor on platelets) with other agonists to increase their platelet activation [8].

Central nervous system serotonin neurons modulate the activity of wide variety of behavioral and neuropsychological processes through multiple 5-HT receptors [9]. Among 5-HT receptors, 5-HT_{2A} is of special concern as it also mediates vascular smooth muscle contraction, platelet aggregation and thrombus formation which are important factors in the development of acute coronary syndromes. It has been suggested that antagonism of this receptor subtype will have a therapeutic benefit in the treatment of cardiac diseases [6].

Several antipsychotic drugs that antagonize 5-HT_{2A} receptor such as clozapine, risperidone and olanzapine have demonstrated some inhibitory effects on platelet aggregation induced by platelet agonists such as collagen, adenosine diphosphate (ADP) and 5-HT in in vitro studies [10-12]. However, in one study clozapine markedly increased platelet aggregation induced by 5-HT [13].

Aim

The aim of the study was to investigate whether SGAs (risperidone, olanzapine and ziprasidone) exert antiplatelet action in the presence of different platelet agonists.

MATERIALS AND METHODOLOGY

Study design

In vitro study

Study subjects

Inclusion criteria:

1- Adult males or females > 18 years and <60,
2- Normal platelet count
3- The individual provided written informed consent to their participation in the research.

Exclusion criteria:

1- Pregnancy.
2- Use of addictive substances (alcohol or opioids).
3- Dental treatment in the previous 14 days to avoid possible gum bleeding.
4- Use of aspirin for primary CAD prophylaxis and/or use of non-steroidal anti-inflammatory drugs in the previous 14 days.

Sample collection

To measure platelet aggregation, we used Multiplate® that uses whole blood sample, rather than platelet rich plasma, to detect platelet aggregation (which resembles physiological condition), and performs a dual measurement to increase accuracy [14]. Blood (3 ml each time) was obtained from 8 adults (convenient sample) (18<age<60 years) healthy volunteers by a certified medical staff at the the Jordan University Hospital (JUH). The blood was collected in hirudin blood vacuumed tube (Double wall), (Verum Diagnostica GmbH, Munich, Germany) where hirudin was used as an anticoagulant, and the whole blood tested within 3 hours from its collection.

Materials

Antipsychotics

Risperidone (10 mg powder)

Olanzapine (10 mg powder)

Ziprasidone (5 mg powder)

Were purchased from (Sigma Aldrich, Germany).

Platelet agonists

Serotonin powder (Sigma Aldrich, Germany), ADP reagent lyophilized (Dynabyte, Munich, Germany), Collagen lyophilized (Dynabyte, Munich, Germany) and Epinephrine solution 1 mg/ml (DEMO S.A. Pharmaceutical Industry, Greece) was purchased from a hospital pharmacy

Preparation of antipsychotics stock concentrations of antipsychotics

As the stock powder of each antipsychotic are contained in amber glass that hold 2 ml of solution, we prepared a stock concentration of 5 mg/ml risperidone and olanzapine by dissolving the 10 mg powder of each one in 2 ml of dimethyl sulfoxide (DMSO), also we prepared a stock concentration of 2.5 mg/ml ziprasidone by dissolving the 5 mg of powder in 2 ml of DMSO.

Preparation of working antipsychotics solutions

We used concentrations of antipsychotics that fall within and/or out of therapeutic ranges (Table 1) [15, 16] to estimate the effect of therapeutic and toxic doses of these antipsychotics. In the beginning, a high toxic concentration above the therapeutic range of each antipsychotic was tested and if a change in platelet aggregation was noticed we test a lower concentration by about one-fourth to one-tenth (according the observed change). A high interval between each consecutive concentration was in order to make it more easily to detect the difference in platelet aggregation between each concentration.

Table 1. Therapeutic ranges and the working concentrations of antipsychotics used in the study.

SGAs	Daily dose (mg/day)	Therapeutic range (ng/ml)	Working concentrations (ng/ml) used
Risperidone	3	4.6	(1.36-1306)
Olanzapine	5-20	8-50	(0.5-522)
Ziprasidone	20-80	20-200	(0.8-726)

One hundred µl of the prepared stock concentrations of each antipsychotic was completed to 1000 µl with DMSO, then diluted with different volumes of normal saline (NS) to prepare different concentrations of each antipsychotic. The highest concentration of DMSO used was 2.7*10-3 V/V (0.27 V/V %).

Four concentrations of each antipsychotic were used, risperidone (1227, 68, 14 and 1.36 ng/ml); olanzapine (491, 54, 5 and 0.5 ng/ml) and ziprasidone (682, 76, 8 and 0.8 ng/ml) to measure the effect of antipsychotics on serotonin-epinephrine combination.

Three concentrations of risperidone (403, 134 and 13 ng/ml), olanzapine (483, 161 and 54 ng/ml) and ziprasidone (672, 224 and 74 ng/ml) were used to measure the effect of these antipsychotics on platelet aggregation induced by 5-HT.

Only one concentration of risperidone (435 ng/ml), olanzapine (522 ng/ml) and ziprasidone (726 ng/ml) was used to measure the effect of these antipsychotics on platelet aggregation induced by epinephrine.

Also one concentration of risperidone (1306 ng/ml), olanzapine (522 ng/ml) and ziprasidone (726 ng/ml) was used to measure the effect of these antipsychotics on platelet aggregation

induced by collagen.

Finally, one concentration of risperidone (1306 ng/ml), olanzapine (174 ng/ml) and ziprasidone (242 ng/ml) was used to measure the effect of these antipsychotics on platelet aggregation induced by collagen.

Stock preparation of agonists

5-HT

To obtain 50 mM of 5-HT, 25 mg powder was dissolved in 2.5 ml of purified water, then swirled gently to mix and allowed to stand at room temperature for 10 minutes.

ADP

To obtain 0.2 mM of ADP, lyophilized ADP was reconstituted with 1.0 ml of purified water, then swirled gently to mix and allowed to stand at room temperature for 10 minutes.

Collagen

To obtain 100 µg/ml, lyophilized collagen was reconstituted with 1.0 ml of purified water, then swirled gently to mix and allowed to stand at room temperature for 10 minutes.

Preparation of working agonists solutions

Serotonin-epinephrine combination

Thirty µl of serotonin solution (50 mM) was added to 30 µl of epinephrine solution (1 mg/ml) then swirled gently to mix and allowed to stand at room temperature for 10 minutes; this 60 µl of agonists combination was added to 300 µl of normal saline (NS) (control) or 300 µl of antipsychotics tested and 300 µl of blood to obtain final 5-HT concentration of 2.27 x 10-3 M and final epinephrine concentration of 2.48 x 10-4 M.

ADP

Twenty mcl of ADP was added to 300 mcl of NS (as a control) or 300 mcl of tested antipsychotics and 300 mcl of blood to obtain a final ADP concentration of 6.5 x 10-6 M.

Collagen

Twenty mcl of Collagen was added to 300 mcl of NS (as a control) or 300 mcl of tested antipsychotics and 300 mcl of blood to obtain a final Collagen concentration of 3.2 µg/ml.

5-HT

Seventy mcl of 5-HT (50 mM) was added to 300 mcl of NS (as a control) or 300 mcl of tested antipsychotics and 300 mcl of blood to obtain a final 5-HT concentration of 5 x 10-3 M.

Epinephrine

Twenty mcl of epinephrine (1 mg/ml) was added to 300 mcl of NS (as a control) or 300 mcl of tested antipsychotics and 300 mcl of blood to obtain a final epinephrine concentration of 5 x 10-3 M.

Normal ranges for AUC (U) induced by different platelet agonists

1. ADP (53-122 U)
2. Collagen (46-117 U) [11].

Measurement of platelet aggregation

Whole blood aggregation was measured using Multiplate® analyzer that detects platelets aggregation based on impedance aggregometry principle. The increase in impedance between the two electrodes contained in the Multiplate® cell due to attachment of platelets onto the Multiplate® sensors is detected and transformed to aggregation units (AU) and plotted against time where the aggregation is quantified as area under the curve (AUC). Multiplate® has three parameters for platelet aggregation measurement, AU, velocity and AUC, the most important parameter being AUC [14]. In this method, as indicated above, 300 µl of whole blood was added to preheated 300 µl NS (control) or a test drug (risperidone, olanzapine or ziprasidone), followed by incubation for 180 seconds, then a platelet aggregation agonist was added and aggregation was continuously recorded for 6 minutes.

Data management and analysis

The percentage of changes in platelet aggregation parameters was assessed for each antipsychotic in comparison to the platelet agonist alone. SPSS (16.0) was used for the statistical analysis to analyze the difference in platelet aggregation (paired t test) between the control and the antipsychotics sample. The level of significance was set at $p < 0.05$.

Clinical difference

The change in aggregation was considered to be clinically significant if it exceeded 20% from control as the internal error of the Multiplate® is reported to be 20% and according to the Multiplate® recommendation in clinical practice this percent is the cut point of the result to be considered significant [14].

Consent

Complying with Declaration of Helsinki, all volunteers were verbally informed about the purpose of the study and that their participation is voluntary, and then they were asked to sign a written informed consent in a simplified language.

Ethical approval

The study started after obtaining Ethical Committee approval from JUH.

RESULTS AND OBSERVATIONS

Effect of DMSO on platelet aggregation induced by different agonists

DMSO alone, at the highest concentration used (2.7 x 10-3 V/V) did not produce any significant inhibition effect (neither clinically nor statistically) on platelet aggregation induced by 5-HT-epinephrine combination (p value = 0.1508).

Also DMSO alone, at the high concentration used (2.9 x 10-3 V/V) did not produce any significant inhibition effect (neither clinically nor statistically) on platelet aggregation induced by neither 5-HT (p value= 0.539) nor epinephrine (p value = 0.1772). Since antipsychotics did show any platelet aggregation inhibition when ADP or collagen were used as agonist, data about DMSO vehicle is not reported.

Effect of platelet agonists on platelet aggregation

Table 2 shows baseline data of the effect of different platelet activators (agonists) on platelet aggregation using Multiplate®. Among all agonists, collagen produced the highest (112 U), whereas 5-HT and epinephrine produced the lowest (19.5 U and 31 U, respectively) platelet aggregation as reflected in AUC. When two platelet agonists, 5-HT and epinephrine, were used in combination, there was a marked amplification of platelet activation compared to each agonist alone, as reflected in AUC (Table 2).

Table 2. Effect of different platelet agonists on aggregation.

Agonist	Concentration (M)	AUC (U) [a]	Average AUC (U) [a]
Collagen	3.2 µg/ml	128.7 96.6	112
ADP	$6.5x10^{-6}$	92.5 87.9 82.2 86.2 105.6 93.9	91
5-HT	$5x10^{-3}$	12.5 13.1 15.5 18.4 29.3 24.5 24.5 23.8	19.5
Epinephrine	$1.76x10^{-4}$	29.7 33.6	31
5-HT with epinephrine	5-HT 2.27 x 10^{-3}	6.6 7.3	7
	epinephrine 2.48 x 10^{-4}	12.7 18.9	15
	5-HT 2.27 x 10^{-3} M with epinephrine 2.48 x 10^{-4}	65.1 68.7	66

[a] 1U corresponds to 10 AU*min.

Effect of antipsychotics on ADP-induced platelet aggregation

As observed from Table 3 neither of antipsychotics at high concentration produced significant effect on ADP-induced platelet aggregation parameters.

Table 3. Effect of antipsychotics on platelet aggregation induced by ADP.

Antipsychotic	Concentration (ng/ml)	Control (U) [a]	Avg. Control (U) [a]	Antipsychotic (U) [a]	Avg. Antipsychotic (U) [a]	% of AUC change	95% confidence interval	P value
Risperidone	1306	92.5 87.9	90	93.2 92.4	93	+3%	-26.7 - 21.5	0.402
Olanzapine	174	82.2 86.2	84	76.6 59.7	68	-19%	-116.7- 148.8	0.367
Ziprasidone	242	105.6 93.9	99	116.6 81.1	98	-1%	-150.3 - 152.1	0.952

[a] 1U corresponds to 10 AU*min

Effect of antipsychotics on collagen-induced platelet aggregation

Table 4 demonstrates that neither of antipsychotics used at high concentration produced significant change in platelet aggregation induced by collagen.

Table 4. Effect of antipsychotics on platelet aggregation induced by collagen.

Antipsychotic	Concentration (ng/ml)	Control (U) [a]	Avg. Control (U) [a]	Antipsychotic (U) [a]	Avg. Antipsychotic (U) [a]	% of AUC change	95% confidence interval	P value
Risperidone	1306	128.7 96.6	112	109.2 99.5	104	-8%	-134.0- 150.6	0.594
Olanzapine	522	128.7 96.6	112	112.4 85.8	99	-13%	-21.4 - 48.5	0.128
Ziprasidone	726	128.7 96.6	112	101.2 97.8	99	-13%	-169.2- 195.5	0.528

[a] 1U corresponds to 10 AU*min

Effect of antipsychotics on 5-HT-induced platelet aggregation

Risperidone and ziprasidone but not olanzapine showed clinically significant inhibition of platelet aggregation induced by by 5-HT, while only ziprasidone showed statistically significant inhibition on 5-HT induce platelet aggregation (Table 5).

Effect of antipsychotics on epinephrine-induced platelet aggregation

All tested antipsychotics showed clinically (but not statistically) significant inhibition on platelet aggregation induced by epinephrine (Table 6).

Effect of antipsychotics on platelet aggregation induced by 5-HT-epinephrine combination

Table 7 demonstrates that all 3 studied antipsychotics decreased AUC platelet aggregation induced by 5-HT-epinephrine combination in a dose-dependent manner (both clinically and statistically significant), this effect disappeared at

low concentrations.

Table 5. Effect of antipsychotics on platelet aggregation induced by 5-HT.

Antipsychotic	Concentration (ng/ml)	Control (U) [a]	Avg. Control (U) [a]	Antipsychotic (U) [a]	Avg. Antipsychotic (U) [a]	% of AUC change	95% confidence interval	P value
Risperidone	403	12.5 13.1	12	5.6 2.3	3	-75%	-15.9 - 33.6	0.138
	134	12.5 13.1	12	10.8 5.7	8	-33%	-31.7 - 40.8	0.356
	13	15.5 18.4	16	7.6 18.4	13	-19%	-46.2 - 54.1	0.500
Olanzapine	483	29.3 24.5	26	29.8 15.9	22	-15%	-53.8 - 61.9	0.537
	161	15.5 18.4	16	16.6 17.9	17	+6%	-10.5 - 9.9	0.772
	54	15.5 18.4	16	28.7 0.1	14	-12%	-197.6-202.7	0.898
Ziprasidone	672	29.3 24.5	26	8.4 5.2	6	-77%	9.935- 30.265	0.025
	224	15.5 18.4	16	13.7 10.1	11	-31%	-36.2 - 46.3	0.364
	74	24.5 23.8	24	30.6 18.3	24	0%	-74.0 - 73.4	0.967

[a] 1U corresponds to 10 AU*min

Table **6**. Effect of antipsychotics on platelet aggregation induced by epinephrine.

Antipsychotic	Concentration (ng/ml)	Control (U) [a]	Avg. Control (U) [a]	Antipsychotic (U) [a]	Avg. Antipsychotic (U) [a]	% of AUC change	95% confidence interval	P value
Risperidone	435	29.7 33.6	31	22.4 21.0	21	-32%	-23.7- 43.6	0.166
Olanzapine	522	29.7 33.6	31	18.4 26.0	22	-29%	-14.1- 33.0	0.123
Ziprasidone	726	29.7 33.6	31	17.6 25.2	21	-32%	-13.3 - 33.8	0.114

[a] 1U corresponds to 10 AU*min

Figure 1: Comparison of percentages of AUC inhibition for antipsychotics on Lineweaver-Burk Plot at three concentrations of the studied antipsychotics

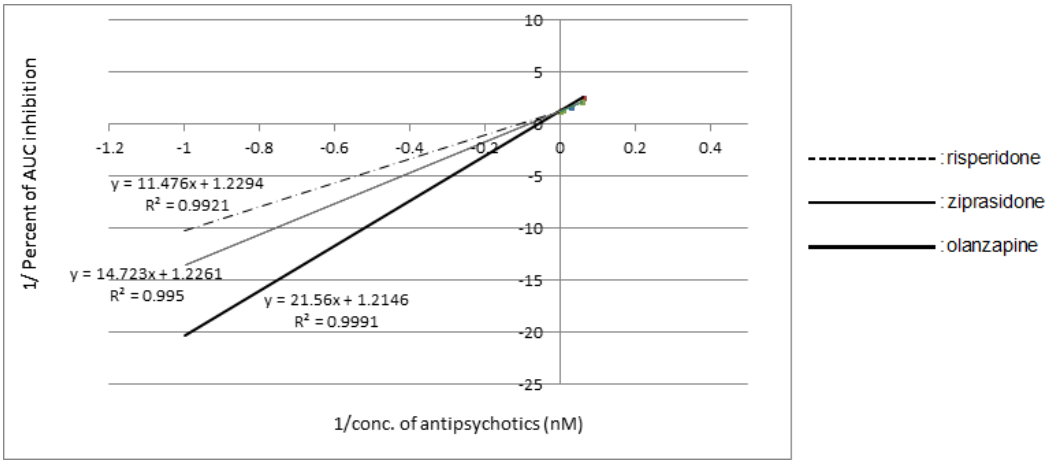

Table 7. Effect of antipsychotics on platelet aggregation induced by 5-HT-epinephrine combination.

Antipsychotic	Concentration (ng/ml)	Control (U) [a]	Avg. Control (U) [a]	Antipsychotic (U) [a]	Avg. Antipsychotic (U) [a]	% of AUC change	95% confidence interval	P value
Risperidone	1227	65.1 68.7	66	10.2 16.7	12	-82% [b]	35.0- 71.9	0.017 [c]
	68	65.1 68.7	66	12.4 19.6	16	-76% [b]	28.0 - 73.8	0.023 [c]
	14	34.1 39.0	36	10.2 16.7	13	-64 [b]	12.9 - 33.3	0.022 [c]
	1.36	34.1 39.9	37	37.5 41.4	39	+5%	-14.5 - 9.6	0.236
Olanzapine	491	65.1 68.7	66	9.4 17.3	13	-80% [b]	26.2 - 80.9	0.026 [c]
	54	65.1 68.7	66	17.9 15.9	16	-76% [b]	14.4 - 85.6	0.036 [c]
	5	30.0 33.6	31	15.0 23.8	19	-39% [b]	-20.6- 45.4	0.132
	0.5	30.0 33.6	31	34.4 34.8	34	+10%	-23.1- 17.5	0.331
Ziprasidone	682	65.1 68.7	66	10.5 12.4	11	-83% [b]	44.7 - 66.2	0.010 [c]
	76	65.1 68.7	66	15.4 19.6	17	-74% [b]	45.6 - 53.2	0.004 [c]
	8	33.0 34.8	33	13.6 21.5	17	-48% [b]	-22.4- 55.1	0.117
	0.8	34.1 39.9	37	40.7 38.9	39	+5%	-51.1- 45.5	0.596

[a] 1U corresponds to 10 AU*min,
[b] clinically significant (change >20%)
[c] statistically significant (P <0.005)

The comparison of the potency and efficacy of antipsychotics

Using four concentrations of each antipsychotic, it was hard to detect difference in efficacy or potency regarding percentage of inhibition of platelet aggregation due to the presence of 0% percent of inhibition (1/0 on y axis) that affects linearity and precision of the relationship between concentration and response.

When we used three concentrations of the studied antipsychotics (deleting the 0% percent to avoid 1/0 on y axis) they showed comparable efficacy but difference in potency with risperidone having the highest, followed by ziprasidone, then by olanzapine (Figure 1).

DISCUSSION

Link between platelets, 5-HT, cardiovascular disorders and antipsychotic medications

Platelets are responsible for pathogenic thrombi formation in patients with atherothrombotic disease, such as ischemic stroke/transient ischemic attack (TIA), ACS, and peripheral artery disease (PAD), and the important role of platelets in such disease is proven by the clinical benefit of antiplatelet medications [8].

Platelet aggregation is the most frequent parameter used to measure platelet function [17], and novelty of our study lies in the fact that the Multiplate® that uses whole blood to measure platelet aggregation has not been used before to investigate the effect of antipsychotics on platelet aggregation. In addition, ziprasidone effect on platelet aggregation has not been tested before by any method.

Platelet aggregation agonists are classified as strong (such as collagen and thrombin) or weak (such as 5-HT and epinephrine) [8], epinephrine sometimes is considered as an intermediate agonist [18]. Our results confirm this view with collagen producing the highest platelet

aggregation AUC (112 U) and 5-HT and epinephrine producing the lowest aggregation AUC (19.5 and 31 U, respectively) although the concentrations of 5-HT and epinephrine used were higher than the concentrations of the other agonists. We used high concentrations of 5-HT and epinephrine as an attempt to induce maximum magnitude of platelet aggregation since these two platelet agonists are regarded as weak.

Activation of 5-HT_{2A} receptor by 5-HT induces platelet aggregation, and thrombus formation and coronary artery spasm. As a result of these actions 5-HT has been linked to vascular and cardiac events especially ischemic heart disease (IHD) [6].

Epinephrine also favors platelet activation in the growing platelet plug. Reduced number of α_{2A}-adrenergic receptors to epinephrine on platelets has been associated with mild bleeding disorders [8], and genetic polymorphism in α_{2A}-adrenergic receptor was associated with increased platelet aggregation induced by epinephrine [19].

Risperidone and ziprasidone but not olanzapine showed clinically significant inhibition of platelet aggregation induced by by 5-HT, while only ziprasidone showed statistically significant inhibition on 5-HT induce platelet aggregation .We were not able to achieve a good linearity between concentrations of risperidone and ziprasidone and their inhibitory effect on 5-HT-induced platelet aggregation due to the fact that 5-HT is only weak platelet agonist (which was reflected by low AUC despite the high concentration we used) and Multiplate® has low sensitivity to detect platelet aggregation at these low AUC that leads to variability in observing accurate magnitude of the effect of antipsychotics on it and prevents establishing good linearity and determining accurate IC50 (although the difference between the AUC % of change produced by the highest concentrations (403 ng/ml of risperidone and 672 ng/ml of ziprasidone) and the lowest concentrations (13 ng/ml of risperidone and 74 ng/ml of ziprasidone) used in this experiment was obvious that indicates a causality effect).

Such low platelet aggregation AUC produced by 5-HT might also explain why olanzapine did not display significant inhibitory (neither clinically nor statistically) effect on 5-HT-induced platelet aggregation despite having high 5-HT_{2A} receptor affinity. However, this affinity is low when compared to risperidone and ziprasidone (ki is 0.2, 0.6 and 3.7 nM for risperidone, ziprasidone and olanzapine, respectively) [5], which made olanzapine effect less visible compared to the other two agents.

We test the effect of antipsychotics on ADP and collagen as widely used platelet agonists, that has been previously tested with antipsychotics with controversial results [10-13]. No significant effect of any antipsychotics (at high concentration) used on platelet aggregation induced by ADP or collagen was observed, this result was expected as no affinity of antipsychotics for ADP purinergic receptors or collagen platelet receptors was shown previously.

Physiologically, platelet aggregation agonists act in combination, and the use of more than one agonist simultaneously better reflects the pharmacodynamics effect of compounds that affect platelets aggregation [18, 20, 21].

Amplification reaction between some agonists may be responsible for the activated state of platelets and the consequences of this activated state are observed in many medical conditions such as IHD, essential hypertension, diabetes mellitus and TIA. Such amplification may reflect the real impact of weak agonists as 5-HT and epinephrine on platelet activity in some thrombotic diseases [18].

Although the synergetic reaction between agonists is a well-known phenomenon [4, 8, 18, 21], a few studies used combination of agonists when investigating the effect of drugs on platelet function [18, 22]. In addition, no study has been evaluated the effect of antipsychotics on platelet aggregation combination before. Significantly amplified platelet aggregation AUC was observed between 5-HT and epinephrine in our study.

Effect of antipsychotics on platelet aggregation induced by combination of 5-HT and epinephrine

Since both 5-HT_{2A} and α_{2A}-adrenergic receptors may be involved in antipsychotics action on the platelets, we investigated the effect of antipsychotics in the presence of the pair of platelet agonists, 5-HT with epinephrine.

We used a lower concentration of both 5-

HT with epinephrine to be closer to physiological concentration, but not low enough because of the low sensitivity of Multiplate® to detect a lower weak agonist concentration. Also to show that the amplification occurs even at lower concentration of the agonist where it appeared that the use of agonist combination is more powerful than the use of high concentration of each agonist alone.

All three antipsychotics inhibited platelet aggregation amplified by 5-HT and epinephrine combination markedly in a dose–dependent manner (both clinically and statistically significant). Although this effect disappeared at low concentrations of olanzapine and ziprasidone, this inhibition effect was consistently significant even at low concentration of risperidone, also risperidone producing the highest inhibitory effect with lowest half maximum inhibitory concentration 50 (IC50= 14.86 nM), followed by ziprasidone (IC50= 19.04 nM), and finally by olanzapine (IC50= 27.56 nM) with high linearity between the closest three concentrations of each antipsychotics and inhibitory effect on aggregation.

The significant inhibitory effect of the antipsychotics on platelet aggregation induced by 5-HT-epinephrine combination is contributed to drug affinity to both 5-HT_{2A} and α_{2A}-adrenergic receptors [18]. That is confirmed in our study where the potency of antipsychotics in inhibition of platelet aggregation induced by 5-HT-epinephrine combination was consistent with their affinity for both 5-HT_{2A} and α_{2A}-adrenergic receptor where risperidone known to have the highest affinity for both 5-HT_{2A} and α_{2A} receptors (ki for 5-HT_{2A} and α_{2A} are 0.2 and 16 nM, respectively) [5], had the lowest IC50 whereas olanzapine, known to have the lowest affinity for both 5-HT_{2A} and α_{2A} receptors (ki for 5-HT_{2A} and α_{2A} are 3.7 and 310 nM, respectively) [5], had the highest IC50.

This inhibition effect was consistently obvious and significant, both clinically and statistically, even at therapeutic range of each antipsychotic (Table 1) [15, 16], although these therapeutic ranges are correlated with moderate doses of the antipsychotics, and according to treatment guidelines for antipsychotics, higher doses are usually used [23].

Also we were interested to investigate the toxic effect of these antipsychotics since overdose of antipsychotics is shown to be quite common [24].

In addition, it has been shown that high doses of antipsychotics are frequently prescribed [25], for example, in Hong Kong high doses are prescribed for 9.2% of the in-patients and 1.8% of out-patients [26]. Also it has been shown that the use of high dose above therapeutic ranges is common [27, 28], where many resistant patients need higher than usual dose ranges to improve their therapeutic outcomes.

So these practices in antipsychotic prescriptions with high doses may have a benefit effect on the risk of ischemic heart diseases in patients taking these antipsychotics but with a possible risk of bleeding. Based on our results, we recommend to monitor bleeding parameters in patients treated with antipsychotics (or other similar drugs) that have a high 5-HT_{2A} and/or α_{2A}-adrenergic receptors affinity, especially at high or toxic doses of such drugs.

Finally, neither 5-HT nor epinephrine has been tested before on Multiplate® and, consequently, no normal ranges for aggregation produced by these agonists have been established before. Thus, our results open the door to the studies of effect on the platelets of compounds that mimic or antagonize the action of weak platelet agonists using Multiplate® and of the normal ranges for platelet aggregation induced by 5-HT and/or epinephrine. However, we suggest the use of further higher concentrations of weak agonists to produce a higher platelet aggregation AUC to enhance the accuracy of results obtained by Multiplate®.

According to our results, it seems that drugs with high affinity for 5-HT_{2A} receptors might be preferred for the treatment of psychiatric comorbid conditions in IHD patients as increased blood 5-HT levels and increased platelet aggregation are associated with cardiac problems such as IHD [7] and as schizophrenic patients are shown to have higher platelet 5-HT levels than healthy individuals [29].

CONCLUSIONS

There was a marked difference in the magnitude of platelet aggregation induced by different activators using Multiplate® with

collagen producing the highest and 5-HT and epinephrine producing the lowest response.

All tested antipsychotics showed clinically (but not statistically) significant inhibition effect on platelet aggregation induced by either 5-HT or epinephrine. None of the studied antipsychotics had an inhibitory effect on platelet aggregation induced by either ADP or collagen.

Significant amplification of platelet aggregation was observed between 5-HT and epinephrine. All antipsychotics studied inhibited platelet aggregation induced by 5-HT-epinephrine combination in a dose-dependent manner, risperidone producing the highest inhibitory effect (the lowest IC50) followed by ziprasidone, and finally olanzapine (the highest IC50).

Our study established new practice of using Multiplate® method for Investigating weak platelet agonists such as 5-HT and epinephrine, evaluating the effect of antipsychotic agents on the platelet aggregation and evaluating the effect of antipsychotics on platelet aggregation induced by platelet agonists combination.

Future study

Future in vivo study should be considered to confirm our results and to correlate the plasma concentration of the antipsychotics with the response

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FINANCIAL DISCLOSURE

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

The authors confirm that the manuscript (or any part of it) has not been published previously and is not under consideration for publication elsewhere.

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