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Full Length Article

In vitro analysis of the effect of contrast agents on the antiaggregant effects of P2Y12 inhibitors

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ABSTRACT

Objectives: The contrast agents have different molarities and ionic structures. The high osmolar contrast agents could increase platelet aggregation but the ionic contrast agents decrease platelet aggregation. However there is insufficient data on whether the antiaggregan effect of P2Y12 inhibitors used during coronary interventions are affected by the contrast agents. This study aimed to evaluate the *in vitro* effects of different contrast agents on the antiaggregant activity of P2Y12 inhibitors (clopidogrel, ticagrelor and prasugrel).

Materials and methods: Thirty patients (who underwent percutaneous coronary interventions and were treated with a P2Y12 inhibitor for a minimum of 10 days) and five healthy volunteers were divided into four groups: the clopidogrel (10 patients), ticagrelor (10 patients), prasugrel (10 patients) and control (five volunteers) groups. Antiaggregant activity was measured by using the Verify-Now method and was represented as P2Y12 reaction unit (PRU) values. Three tubes of blood were collected from the participants in the three patient groups and in the control group; as the contrast material, 10% iohexol was added to a second tube, and 10% iodixanol was added to a third tube. PRU values of the control and the contrast tubes were measured at 5 min and at 30 min after the contrast material was added.

Results: Iohexol and iodixanol led to a significant decrease in the PRU values in the control group (iohexol: 188.4 ± 39.2 *vs* 142.4 ± 17.0, $p = .04$; iodixanol: 188.4 ± 39.2 *vs* 159.2 ± 33.7, $p = .04$) and in the clopidogrel group (iohexol: 140.8 ± 50.8 *vs* 106.3 ± 44.4, p = .04; iodixanol: 140.8 ± 50.8 *vs* 109.4 ± 47.6, $p = .009$) but not in the ticagrelor and prasugrel groups. The PRU values were significantly lower in the ticagrelor (23.1 ± 26.2) and prasugrel (23.4 ± 27.5) groups than in the clopidogrel (140.8 ± 50.8) and control (188.4 ± 39.2) groups ($p < .01$), and the PRU values for the ticagrelor and prasugrel groups were similar for both the 5-min and 30-min time periods ($p > .05$). The antiaggregant activities of iohexol and iodixanol were observed to be similar at the 5- and 30-minute time points for all of the groups ($p > .05$).

Conclusion: Iohexol and iodixanol had *in vitro* antiaggregant effects, and their antiaggregant effects were similar. Iohexol and iodixanol increased the clopidogrel antiaggregant activity *in vitro,* but they did not significantly alter the antiaggregant activities of prasugrel and ticagrelor.

1. Introduction

During coronary angiography, a contrast agent is used to visualize the vessels. Contrast agents have different properties that are based on their osmolarities (high osmolarity, low osmolarity or isoosmolarity), on their single- or double-chain chemical structures (monomeric or dimeric) and on their ionization statuses (ionized or nonionized) (1). Iso-osmolar or low osmolar contrast agents reduce the risks of nephropathy and allergic reactions, and they have been shown to have a variety of effects on platelet aggregation in *in vitro* experiments ([2–5]. The ionicity of the contrast agent gives it its antiaggregant properties (2).

The presence of resistance to P2Y12 inhibitors, or the insufficient antiaggregant effects of these agents, may lead to complications, such as stent thrombosis and acute myocardial infarction (6–9). Although previous studies have reported an interaction between clopidogrel and contrast agents, there are no data demonstrating whether new P2Y12 inhibitors (ticagrelor and prasugrel) interact with contrast agents (10).

This study aimed to conduct an *in vitro* evaluation of whether there was any interaction between the contrast agents that we use during the balloon stent procedures for coronary artery patients and the P2Y12 inhibitors (clopidogrel, ticagrelor and prasugrel) that we use to treat these patients.

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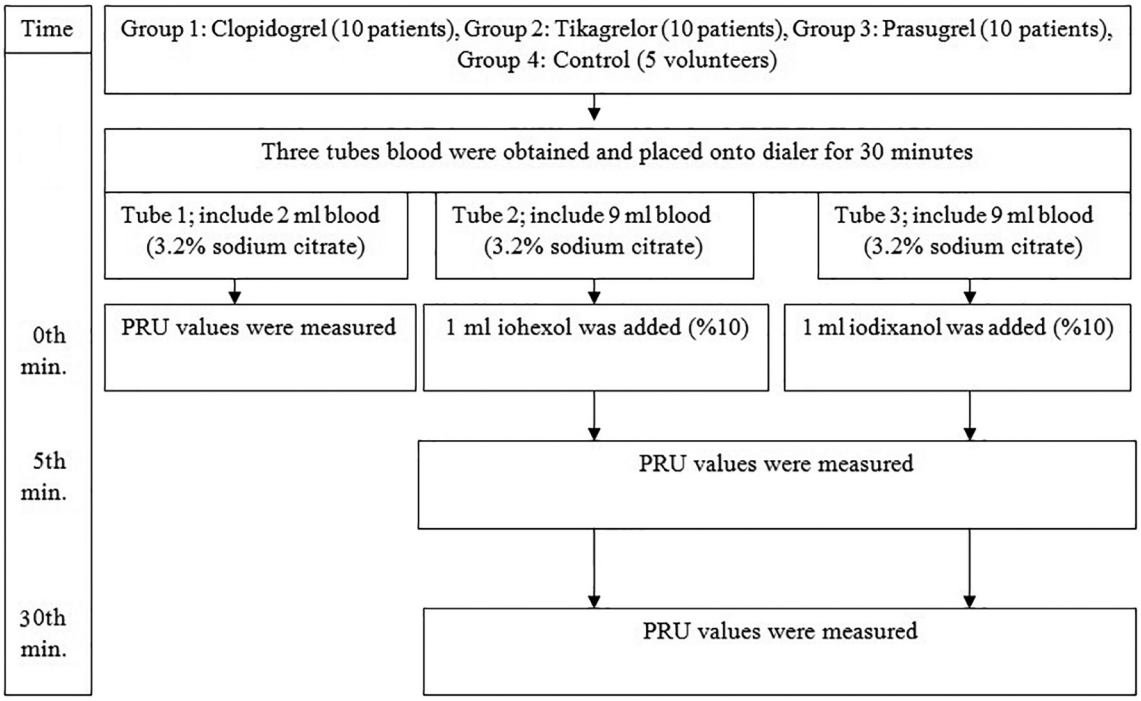

Fig. 1. Study design. 0 th min. represent baseline measurement.

2. Method

The study began after approval was obtained from the local Ethics Committee. Patients who had been diagnosed with low risk acute coronary syndrome (ACS) between the ages of 18 to 75 years and who had been admitted to the cardiology clinic between November 25th, 2017 and February 1st, 2018 were prospectively evaluated. Patients were randomized into the clopidogrel, ticagrelor and prasugrel treatment groups. Standard clinical doses of these drugs that are used in clinical settings were used in this study. The process of patient randomization into the treatment groups (75 mg 1 × 1 clopidogrel, 90 mg 2 × 1 ticagrelor and 10 mg 1 × 1 prasugrel) was automatically accomplished by the use of a computer program. Patients receiving P2Y12 inhibitor (clopidogrel, ticagrelor or prasugrel) treatments for at least 10 days were evaluated for P2Y12 resistance, and the patients with PRU values that were > 208 were excluded from the study. A maximum of 10 patients were included in each group (clopidogrel, prasugrel and ticagrelor). Individuals who had presented to the cardiology clinic in the same time period with atypical complaints, without the presence of cardiovascular risk factors (except for cigarette smoking) and with similar ages among the study groups were evaluated. Five volunteers with normal ECG, transthoracic echocardiography and biochemical laboratory tests were automatically randomized into the control group by the use of a computer program. Volunteers were not using any drugs, and no new drugs were prescribed, during the study period. Informed written consent was obtained from each of the participants (a total of 30 patients and 5 volunteers), in accordance with the study protocol that was approved by the local Ethics Committee.

The demographic characteristics of the patients were recorded, and physical examinations were performed. The baseline biochemical values and risk factors were recorded, and blood pressure (BP) was measured before the procedures. Patients with systolic blood pressure (SBP) values ≥140 mm Hg and/or diastolic blood pressure (DBP) values ≥90 mm Hg, as well as patients who were taking antihypertensive drugs, were defined as being hypertensive. Patients were defined as being diabetic if the fasting blood glucose levels were ≥126 mg/dL in two consecutive measurements, or if they were using oral antidiabetics/insulin. A family medical history was obtained from each of the patients, and they were also asked if they were smokers. The heights and weights of the patients were measured. Body mass index (BMI) values were calculated by using the following formula: weight (kg)/square of the height (m^2).

The 35 patients in the study were divided into the following four groups: Group 1, patients using clopidogrel (10 patients); Group 2, patients using ticagrelor (10 patients); Group 3, patients using prasugrel (10 patients) and Group 4, the control group (five volunteers).

The following contrast agents were used: low osmolarity nonionic iohexol (350 mg I/ml) and isoosmolar nonionic iodixanol (320 mg I/ml).

The VerifyNow-P2Y12 test cartridge system (Accumetrics, San Diego, CA, USA) is a rapid (< 5 min) platelet function assay designed to measure directly the effects of drugs on the P2Y12 receptor. The VerifyNow-P2Y12 assay uses prostaglandin E1 in addition to ADP to increase intraplatelet cAMP, making the assay more sensitive and specific for the effects of ADP mediated by the P2Y12 receptor. The whole-blood citrate mixture is added to the cartridge, and agglutination between platelets and coated beads is recorded. The VerifyNow-P2Y12 instrument is a turbidimetric optical detection system, which measures platelet induced aggregation as an increase in light transmittance. VerifyNow-P2Y12 assay results are expressed in P2Y12 reaction units (PRU). The antiaggregant characteristics of clopidogrel, ticagrelor and prasugrel were evaluated by using the Verify-Now method. The PRU values were calculated, and the antiaggregant activity was measured by using the method reported in previously (11).

3. Study procedure

Two hours after the last drug dose (clopidogrel, prasugrel or ticagrelor), three tubes (3.2% sodium citrate tubes) of blood were obtained from both the patients and the healthy controls in the blood collection section of the biochemistry laboratory. The first tube was a 2 ml blood collection tube (2 ml sodium citrate tube, GongDong) and the second and third tubes were 10 ml blood collection tubes (10 ml sodium citrate tube, GongDong). All of the tubes had a 3.2% sodium citrate to whole blood ratio of 10%. A total of 2 ml of blood was added to the first tube, and 9 ml of blood was added to the other two tubes. All of the blood

Table 1
Demographic and laboratory findings of study groups.

Study group	Clopidogrel (n = 10)	Ticagrelor (n = 10)	Prasugrel (n = 10)	Control (n = 5)	P value
Age, year	54,3 ± 11	52,8 ± 9,9	50,8 ± 9,3	39,4 ± 9,4	0,08
Gender, n (%)	10	10	10	5	0,46
Male	9 (90)	10 (100)	10 (100)	5 (100)	
Female	1 (10)	–	–	–	
Body mass index, (kg/m^2)	27,2 ± 3,3	28,9 ± 3,9	26 ± 2,6	24,7 ± 3,2	0,18
Hypertension, n (%)	2 (20)	1 (10)	1 (10)	–	0,70
Diabetes mellitus, n (%)	3 (30)	3 (30)	3 (30)	–	0,56
Heredity, n (%)	3 (30)	2 (20)	3 (30)	–	0,55
Hyperlipidemia, n %	5 (50)	2 (20)	1 (10)	–	0,08
Cigarette, n (%)	4 (40)	7 (70)	9 (90)	3 (30)	0,12
EF, %	**40,5 ± 8,9**	**41,5 ± 8,5**	**46 ± 9,6**	**62,0 ± 3,1**	**0,004**
Hemoglobin (gr/dL)	13,3 ± 2	13,4 ± 1,6	14,8 ± 1,3	14,5 ± 0,7	0,09
Platelet (K/μL)	290,7 ± 75,9	217,1 ± 74,4	268,4 ± 65,4	274,6 ± 44,4	0,08
Creatinin (mg/dL)	0,91 ± 0,18	0,92 ± 0,12	0,82 ± 0,2	0,73 ± 0,19	0,16
CRP (mg/dL)	11 ± 8,2	4,1 ± 2,4	5,6 ± 3,6	4,1 ± 2,3	0,13

EF: Ejection fraction, CRP: C-Reactive Protein.

tubes were placed onto the dialer for 30 min. After 30 min, 1 ml of iohexol was added to the second tube, and 1 ml of iodixanol was added to the third tube. Ten percent contrast agents were added to the second and third tubes (this percentage represents approximately 500 ml of contrast agent in real life procedures). Five minutes after the addition of the contrast agents, the PRU values were measured in the control tube and the tubes to which the contrast media had been added. At the 30-minute time point, the PRU values were again calculated by measuring the blood samples from the 2nd tube and the 3rd tube, which both contained the added contrast media (Fig. 1).

The PRU values were calculated by using the Verify-Now test (ADP kit) (Accumetrics Ltd., San Diego, California) in the biochemistry laboratory by operators trained and experienced in PRU measurement. The evaluation results were recorded, and the effects of the contrast agents on the P2Y12 inhibitors were compared between the control and the contrast agents.

Group 1: the clopidogrel group: 10 patients who received 75 mg 1 × 1 clopidogrel for > 10 days; blood was obtained from each of the patients (three tubes).

Tube 1 (empty): PRU values were measured; tube 2 (10% iohexol was added): PRU values were measured at the 5- and 30-minute time points; tube 3 (10% iodixanol was added): PRU values were measured at the 5- and 30-minute time points.

Group 2: the ticagrelor group: (10 patients who received 90 mg 2 × 1 ticagrelor for > 10 days); blood was obtained from each of the patients (three tubes).

Tube 1 (empty): PRU values were measured; tube 2 (10% iohexol was added): PRU values were measured at the 5- and 30-minute time points; tube 3 (10% iodixanol was added): PRU values were measured at the 5- and 30-minute time points.

Group 3: the prasugrel group: (10 patients who received 10 mg 1 × 1 prasugrel for > 10 days); blood was obtained from each of the patients (three tubes).

Tube 1 (empty): PRU values were measured; tube 2 (10% iohexol was added): PRU values were measured at the 5- and 30-minute time points; tube 3 (10% iodixanol was added): PRU values were measured at the 5- and 30-minute time points.

Group 4: the control group (5 volunteers); blood was obtained from each of the volunteers (three tubes).

Tube 1 (empty): PRU values were measured; tube 2 (10% iohexol was added): PRU values were measured at the 5- and 30-minute time points; tube 3 (10% iodixanol was added): PRU values were measured at the 5- and 30-minute time points.

The patients in the control group did not undergo any procedure, other than ECG, transthoracic echocardiography and biochemical tests. No treatments were prescribed to the control group.

For the sample sizes, estimated mean changes for platelet aggregation were obtained from the published literature (10). In the study, platelet aggregation was evaluated with the use of the PFA-100 analysis system. Compared to saline, the membrane clotting time (an anti-aggregant effect) was increased by 48% in the iohexol group and by 55% in the iodixanol group (10). We calculated the number of patients who would be necessary to detect differences in the PRU values of 40 ± 30 between the control and contrast agent groups, with an α of 0.05, a β of 0.20 and a statistical power of 0.80. The necessary number of patients for each of the groups was 8 individuals. Therefore, we enrolled 10 patients into each group.

Statistical analyses were performed by using the SPSS program (Statistical Package for the Social Sciencesver. 23, SPSS Inc., Chicago, Illinois, USA). The continuous variables were reported as the means ± standard deviations, and the categorical variables were reported as percentages. To compare the means between the groups, a Student's *t*-test was used for the comparison of the normally distributed variables, and a Mann–Whitney *U* test was used for the comparison of the nonnormally distributed variables. A Kruskal-Wallis test was used for the comparison of the continuous variables of the baseline characteristics of the four groups. A chi-square test or a Fisher's exact test were used for the comparison of the categorical variables of the baseline characteristics of the four groups. A Wilcoxon Sign Rank test was used to evaluate the repeated measurements. A P value of < 0.05 was considered to be statistically significant in all of the evaluations.

4. Results

4.1. Patient characteristics

A total of 35 patients were included in the study, and the mean age was 50.7-years-old. Of the patients, 97.1% (34) were male, and 2.9% (1) were female. The mean BMI was 27.03 (kg/m^2). Hypertension was present in 11.4% (4) of the patients, with 22.9% (8) of the patients being hyperlipidaemic and 25.7% (9) being diabetic.

When the included patients in the study were evaluated in terms of the demographic characteristics, the ejection fraction of the volunteers in the control group was higher than that of the other groups (p = .004) (Table 1). In terms of the other parameters, all of the groups were similar.

4.2. PRU values with iohexol and iodixanol

The initial PRU values were 188.4 ± 39.2 for the control group, 140.8 ± 50.8 for the clopidogrel group, 23.1 ± 26.2 for the ticagrelor group and 23.4 ± 27.5 for the prasugrel group. The PRU values that

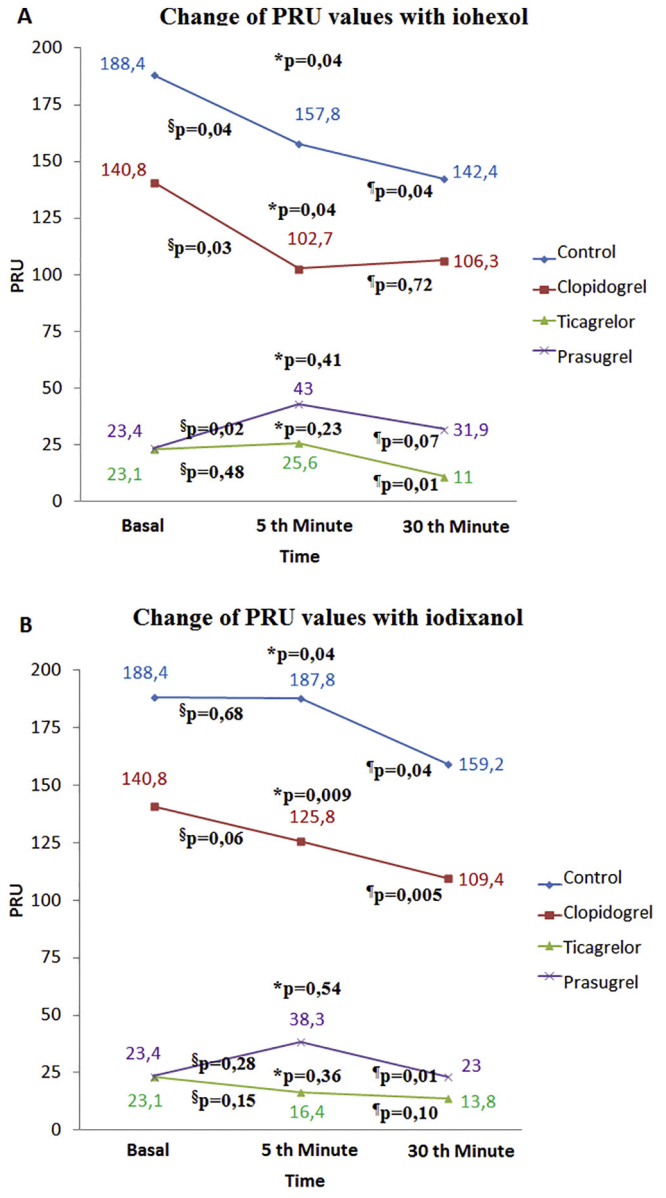

Fig. 2. a. Changes in PRU values with iohexol, **2B**: Changes in PRU values with iodixanol.
§: basal and 5-minute comparison, ¶: 5- and 30-minute comparison, *: Basal and 30-minute comparison.

were measured at 5 min after the addition of iohexol into the tubes were 157.8 ± 22.1 for the control group, 102.7 ± 52.7 for the clopidogrel group, 25.6 ± 25.9 for the ticagrelor group and 43.0 ± 28.2 for the prasugrel group. There were significant decreases in the PRU values that were measured at the 5-minute time points in the iohexol-added tubes in the control group ($p = .04$) and in the clopidogrel group ($p = .03$), there was no significant change in the ticagrelor group ($p = .48$), and there was a significant increase in the prasugrel group ($p = .02$) (Fig. 2A). The PRU values that were measured at 30 min after the addition of iohexol were 142.4 ± 17.0 for the control group, 106.3 ± 44.4 for the clopidogrel group, 11.0 ± 15.6 for the ticagrelor group and 31.9 ± 26.9 for the prasugrel group. Compared to the measurements at the 5-minute time points, there were significant decreases in the PRU values at the 30-minute time points in the control group ($p = .04$) and in the ticagrelor group ($p = .01$), whereas there were no significant changes in the clopidogrel ($p = .72$) or prasugrel ($p = .07$) groups (Fig. 2A). When compared to the baseline measurements, significant decreases were observed in the PRU values in the control group (p = .04) and in the clopidogrel group (p = .04) at the 30-minute time points, whereas there were no significant changes in the ticagrelor ($p = .23$) and prasugrel ($p = .41$) groups (Fig. 2A).

The PRU values that were measured at 5 min after the addition of iodixanol to the tubes were 187.8 ± 34.6 for the control group, 125.8 ± 49.3 for the clopidogrel group, 16.4 ± 22.9 for the ticagrelor group and 38.3 ± 28.8 for the prasugrel group. There were no significant changes in the PRU values that were measured at the 5-minute time points in the iodixanol-added tubes in the control group ($p = .68$), in the clopidogrel group ($p = .06$), in the ticagrelor group ($p = .15$) and in the prasugrel group ($p = .28$) (Fig. 2B). The PRU values that were measured at 30 min after the addition of iodixanol were 159.2 ± 33.7 for the control group, 109.4 ± 47.6 for the clopidogrel group, 13.8 ± 19.0 for the ticagrelor group and 23.0 ± 26.9 for the prasugrel group. When compared to the measurements at the 5-minute time points, there were significant decreases in the PRU values at the 30-minute time points in the control group ($p = .04$), in the clopidogrel group ($p = .005$) and in the prasugrel group ($p = .01$), whereas there were no significant changes in the ticagrelor group ($p = .10$) (Fig. 1B). When compared to the baseline measurements, significant decreases were observed in the PRU values in the control group (p = .04) and in the clopidogrel group ($p = .009$) at the 30-minute time points, whereas there were no significant changes in the ticagrelor ($p = .36$) and prasugrel ($p = .54$) groups (Fig. 2B).

Both iohexol and iodixanol exhibited significantly lower PRU values at the baseline, 5-minute and 30-minute time points in the clopidogrel, ticagrelor and prasugrel groups than in the control group (Table 2). During the administration of both contrast agents, the PRU values at baseline, 5-minute and 30-minute time points were again observed to be significantly lower in the ticagrelor and prasugrel groups, compared to the clopidogrel group (Table 2). In terms of the PRU values, there were no significant differences between iohexol and iodixanol administrations in patients with ticagrelor and prasugrel (Table 2).

Table 2
Comparison of PRU values between control and P2Y12 inhibitors group.

	Clopidogrel (n = 10)	Ticagrelor (n = 10)	Prasugrel (*n* = 10)	Control (n = 5)	Cl *vs* C	Cl *vs* T	Cl *vs* P	T *vs* C	P *vs* C	T *vs* P
Basal	140,8 ± 50,8	23,1 ± 26,2	23,4 ± 27,5	188,4 ± 39,2	0,04	<0,01	<0,01	<0,01	<0,01	0,98
Iohexol 5th minute	102, 7 ± 52,7	25,6 ± 25,9	43,0 ± 28,2	157,8 ± 22,1	0,01	<0,01	<0,01	<0,01	<0,01	0,16
Iohexol 30th minute	106,3 ± 44,4	11,0 ± 15,6	31,9 ± 26,9	142,4 ± 17,0	0,04	<0,01	<0,01	<0,01	<0,01	0,06
Iodixanol 5th minute	125,8 ± 49,3	16,4 ± 22,9	38,3 ± 28,8	187,8 ± 34,6	0,01	<0,01	<0,01	<0,01	<0,01	0,07
Iodixanol 30th minute	109,4 ± 47,6	13,8 ± 19,0	23,0 ± 26,9	159,2 ± 33,7	0,04	<0,01	<0,01	<0,01	<0,01	0,39

C: Control, Cl: Clopidogrel, P: Prasugrel, T: Ticagrelor.

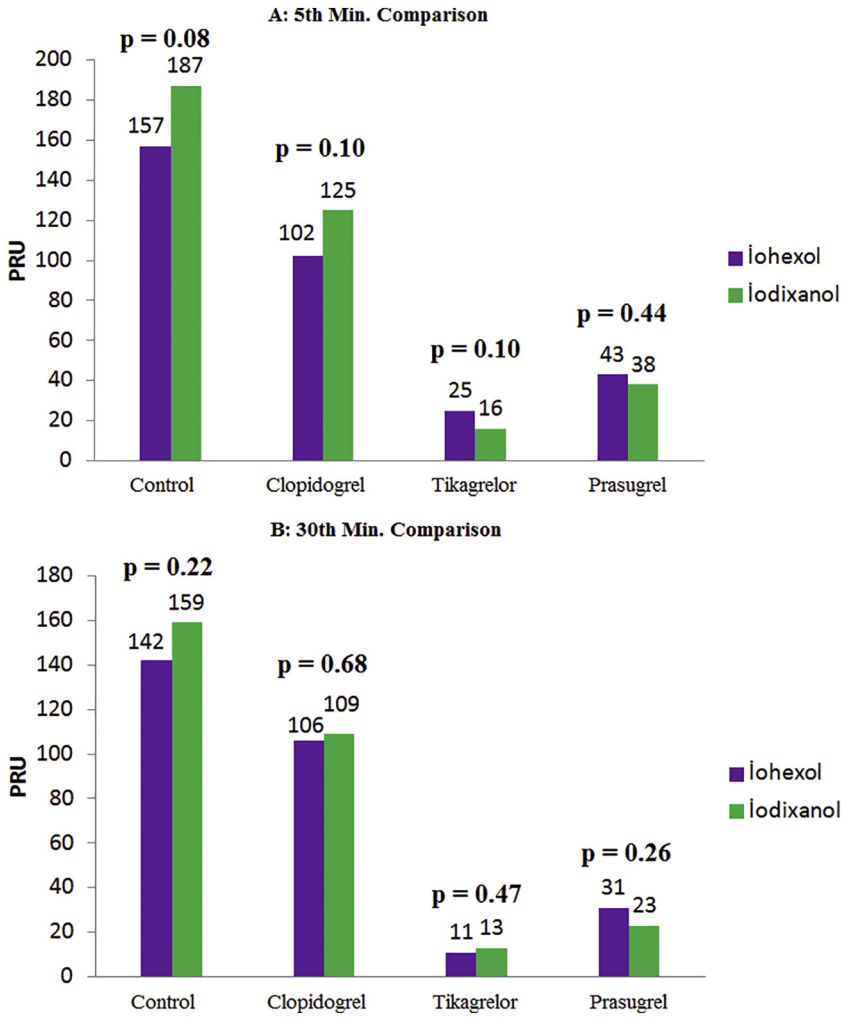

Fig. 3. Comparisons of the effects of iohexol and iodixanol on PRU values at 5-and 30-minute time points.

The PRU values of the control, clopidogrel, ticagrelor and prasugrel groups at 5 min after the administration of iohexol and iodixanol were similar (Fig. 3). Additionally, the PRU values of all groups at 30 min after the administration of both contrast agents were similar (Fig. 3).

5. Discussion

The main results of our study are as follows: a) iohexol and iodixanol had *in vitro* antiaggregant activities; b) iohexol and iodixanol had similar *in vitro* antiaggregant activities, but the antiaggregant activity of iohexol occurred more quickly; c) iohexol and iodixanol increased the *in vitro* antiaggregant activity of clopidogrel; d) iohexol and iodixanol exhibited a slight reduction in the *in vitro* antiaggregant activity of prasugrel in the early time period, but they did not exhibit a significant difference at the 30-minute time point; e) iohexol and iodixanol did not exhibit any significant changes in the *in vitro* antiaggregant efficacy of ticagrelor in the early time period, but iohexol and iodixanol slightly increased the *in vitro* antiaggregant activity of ticagrelor at the 30-minute time point and f) the *in vitro* antiaggregant activities of ticagrelor and prasugrel in the presence of iohexol and iodixanol were significantly higher than that of clopidogrel at the baseline, 5-minute and 30-minute time points.

In our study, contrast agents (low osmolar nonionic iohexol and isoosmolar nonionic iodixanol) exhibited antiaggregant effects by decreasing the PRU values in the control group, which did not receive P2Y12 inhibitors. This antiaggregant effect started in the early time period and continued into the late time period in the iohexol group, whereas the antiaggregant effect occurred in the late time period in the iodixanol group. In a previous study, the results were consistent with our data (12). The early emergence of the antiaggregant activity of iohexol (in monomeric form, it contains a higher concentration of iodine), with respect to iodixanol (with a dimeric structure, it contains a lower concentration of iodine), may be due to the structural and osmolality differences between the two agents.

In our study, nonionic hypo-osmolar and isoosmolar agents were used in the control group and in the P2Y12 inhibitor groups, and these agents demonstrated similar antiaggregant effects at 5 and 30 min. A similar effect may indicate that the main effects of the contrast agents on platelet aggregation may be due to their being either ionic or nonionic. In addition, the antiaggregant substances in the testing environment for the measurement of aggregation can also affect the test results. Aggregation tests in citrate-containing tubes and in hirudin-containing tubes may produce different results between the hypo-osmolar and isoosmolar agents (12). In our study, citrate was used as the antiaggregant at the same concentrations in all of the tubes. There is also a need to retest the efficacies of these two agents *in vivo*.

In our study, the PRU values of the patients who used both ticagrelor and prasugrel were significantly lower than the PRU values of patients who used clopidogrel. The *in vitro* antiaggregant properties of these drugs (prasugrel and ticagrelor) were significantly higher than that of clopidogrel. Many studies that compared the antiaggregant effects of prasugrel and ticagrelor with clopidogrel have shown that these two agents are more effective than clopidogrel. However, it was shown for the first time in our study that the effectiveness of these two agents

is higher than clopidogrel in the presences of iohexol and iodixanol. In our study, the effectiveness of these agents was demonstrated in an *in vitro* setting. Therefore, it is necessary to confirm these effects with *in vivo* studies.

There were no significant changes in the antiaggregant activities of ticagrelor and prasugrel with the additions of iohexol and iodixanol. This may be because of the maximal antiaggregant activities that were achieved with these two agents. However, in the group of patients receiving clopidogrel, iohexol and iodixanol caused an increase in the antiaggregant activity of clopidogrel. In a previous study, it was demonstrated that iopamidol and iodixanol were effective in increasing platelet aggregation by the addition of ADP and PAF to the environment (12). In our study, iohexol and iodixanol increased antiaggregant activity in the presence of clopidogrel.

In our study, patients with P2Y12 resistance were excluded. The real effects of three drugs were examined. The PRU values of the clopidogrel group were higher than in the other two drug groups. This is related to the higher antiaggregant activities of ticagrelor and prasugrel than clopidogrel (13). By increasing the dose of clopidogrel, these drugs can be comparable at similar PRU values, but this does not reflect the real-life settings (14). In our study, the real-life doses of drugs were evaluated.

In an *in vivo* study on rats, there was no increase in the activity of clopidogrel with iohexol (a nonionic contrast agent) administration; however, the efficacy of clopidogrel was increased with the ioxaglate (an ionic contrast agent) administration (15). Therefore, the findings of our study require confirmation in an *in vivo* study.

When iohexol was added to the environment, prasugrel PRU values were increased significantly at the 5th minute compared to baseline and returned to baseline levels at the 30th minute. Although not significant, a similar effect is observed with the addition of iodixanol. This time-based change was not seen in clopidogrel and ticagrelor groups. This finding may be due to the pharmacological properties of prasugrel (16).

The effects of contrast agents on platelet function are clinically important. Although all of the contrast agents have antiaggregant properties, the ionic agents clearly have more potent antiaggregant effects than the nonionic agents. In a study, ioxaglate (an ionic and low osmolar contrast agent) has been shown to inhibit thrombin-induced platelet activation (17). In a meta-analysis, ioxaglate was shown to decrease the rate of acute coronary artery occlusion by 32%, compared with the nonionic contrast agents ($p = .03$) (18).

As previously described, contrast agents interact with thrombolytic drugs *via* platelet activation and degranulation, thus resulting in changes in the coagulation mechanism. The fact that the contrast agent can be either isoosmolar or hypo-osmolar is not very important, in terms of its effect on aggregation; the main point is that the contrast agent is either ionic or nonionic (10). Ionic contrast agents inhibit both intrinsic and extrinsic coagulation cascades at various stages. The ionic contrast agents act as direct inhibitors of thrombin production. They also inhibit both platelet activation and platelet aggregation, increase bleeding time and inhibit fibrinolysis enzymes. The ionic contrast agents increase clotting time by approximately four times, compared to nonionic contrast agents (10). Nonionic contrast agents interact with the coagulation mechanism by inhibiting the coagulation cascade after thrombin formation in the fibrin monomer polymerization step; however, the nonionic agents exhibit less interaction than that of the ionic agents (19, 20). Therefore, both ionic and nonionic agents may increase the clotting time and may increase the effects of anticoagulants and antiplatelet drugs (10).

In previous studies, different contrast agents were evaluated by different bleeding and coagulation tests. The results of these studies are in favor of the fact that contrast agents do not cause serious thrombus formations during PCI. However, it has been shown in several studies that thrombi or aggregates were formed on the catheter and guidewires, especially during prolonged coronary angiography in patients who did not receive anti-aggregants or anticoagulants (21).

The contrast agent ratio that was used in our study was 10%. This corresponds to approximately 500 ml of contrast agent that is used during coronary procedures. This amount is higher than the amount of contrast that is used in standard procedures; however, in chronic total occlusion interventions, contrast agents are applied at these doses. The impacts of contrast agents on the antiaggregant effects of drugs (P2Y12 inhibitors) may not be fully evident with low doses. Therefore, high doses of the contrast concentration ratio were evaluated in our study.

According to our study *in vitro* setting, contrast agents were increasing the antiaggregant effects of clopidogrel. However, antiaggregant activity of clopidogrel was still less than prasugrel and ticagrelor. In clinical trials, major bleeding (fatal, intracranial, bleeding that requires pressors or surgery, bleeding with decrease in hemoglobin > 5 g/dL,requiring transfusion of ≥ 4 U) with ticagrelor which has a stronger antiaggregant effect, was similar to clopidogrel (22). In this respect, our findings suggest that iohexol and iodixanol would be unlikely to significantly increase in the bleeding risk of clopidogrel.

If confirmed by *in vivo* studies, the clinical effects that our study results may have can be listed as follows. In our study, it was found that contrast agents (iohexol, iodixanol) coused increase in antiaggregant activity. Therefore, it may be argued that contrast agents used during angiographic or diagnostic procedures may lead to an increase in bleeding complications in patients at high risk for bleeding. On the other hand, contrast agents may have a beneficial effect by increasing antiaggregant activity, especially in patients receiving clopidogrel, especially in conditions such as acute coronary syndrome where a strong antiaggregant effect is needed. These contrast agents may increase the anti-thrombotic properties of clopidogrel, thereby closing the gap between clopidogrel and ticagrelor/prasugrel, which are more potent P2Y12 inhibitors. Iohexol and iodixanol could potentially affect the balance between adequate prevention of thrombosis and unwanted bleeding in patients undergoing PCI. However clinical implications are uncertain until findings are confirmed in a *in vivo* clinical study.

The extrinsic factors that occur during coronary angiography and intervention are one of the most important steps in the onset of coagulation. Differences may occur between *in vitro* and *in vivo* experiments because such factors cannot be obtained in *in vitro* experiments. However, studies that have been conducted *in vitro* can provide foundations for *in vivo* studies.

In conclusion, iohexol and iodixanol had antiaggregant activities in an *in vitro* setting, and the antiaggregant activities of these agents were similar. It was observed that these two contrast agents increased the antiaggregant activity of clopidogrel *in vitro* but did not significantly change the antiaggregant activities of prasugrel and ticagrelor. The antiaggregant activities of ticagrelor and prasugrel were significantly higher than that of clopidogrel. The results of our study that tested the antiaggregant activities of iohexol and iodixanol should be confirmed by *in vivo* trials.

Declaration of competing interest

The authors declare no conflicts of interest.

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