Impact of Calcium Channel Blockers on Aspirin Reactivity in Patients with Coronary Artery Disease

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Abstract

Purpose Calcium channel blockers (CCBs) do not reduce the risk of initial or recurrent myocardial infarction (MI) in patients diagnosed with stable coronary artery disease (CAD). The aim of this current study was to evaluate the association between CCBs and aspirin resistance in patients with CAD.

Methods Patients with stable CAD who were regularly taking aspirin (75–100 mg qd) for at least 1 month prior to enrollment in the study were included. The VerifyNow system was used for platelet function testing with high on-aspirin platelet reactivity (HAPR) defined as aspirin reaction units (ARU) >550. We compared patients treated with CCBs versus control group.

Results Five hundred three patients with CAD were included in this study, and 88 were treated with CCBs. Mean age (67.9±9.7 in the CCB group vs. 66.5±11.4 in the control group), gender (77.3 male vs. 82.9%), rates of diabetes mellitus (34.7 vs. 36.9%), rates of CKD (23.5 vs. 23.5%), dyslipidemia (85.1 vs. 85.3%), and statin therapy (89.5 vs. 90.7%) were similar. The mean ARU was 465.4±70.0 for patients treated with CCBs versus 445.2±60.0 in controls (p=0.006). Similarly, 15.9% of CCB patients demonstrated HAPR compared to 7.0% (p=0.006). The administration of CCBs was independently associated with HAPR in a multivariate analysis (OR 1.72, 95% CI: 1.04–8.91, p=0.047) as well as in propensity score matched analysis (OR 1.56; CI: 1.22–1.93; p<0.001).

Conclusions Usage of CCBs is positively correlated with aspirin resistance. These findings may suggest an adverse pharmacologic effect of CCBs among patients with stable CAD treated with aspirin.

Keywords Calcium channel blockers · High on-aspirin platelet reactivity · Coronary artery disease · Acute coronary syndrome

Introduction

In platelets, one of the pivotal activating factors that leads to aggregation occurs through the messaging cascade initiated by calcium influx [1]. Initially, agonists like subendothelial collagen, thromboxane A2 (TXA2), and ADP released from platelets all contribute to an intracellular increase in calcium storage. As the calcium concentration rises within the cell, the signaling cascade leads to both actin cytoskeleton reorganization and degranulation, which are essential for platelet aggregation [1–3]. Aspirin prevents these steps and halts platelet aggregation. It inhibits the activation of arachidonic acid, thereby preventing TXA2 from causing a rise in intracellular calcium [4]. However, the effects of aspirin are dependent on many factors. Nearly a quarter of patients who have been prescribed aspirin for prevention of cardiac or cerebral vascular events have an inadequate response to the drug [5, 6]. This decreased response may stem from many sources, including increased levels of epinephrine, which can inhibit aspirin’s effectiveness and reverse its anti-thrombotic effect [7, 8]. Similarly, increased levels of homocysteine in the plasma are associated with augmented aspirin resistance among patients with coronary artery disease (CAD) [9, 10]. Aspirin resistance can
be assessed using quantitative measures, including aspirin reaction units (ARU) and high on-aspirin platelet reactivity (HAPR). HAPR can be a result of both pharmacokinetic and pharmacodynamic properties. Pharmacokinetic mechanisms include medication nonadherence, decreased bioavailability, and drug-drug interactions with other COX inhibitors. Pharmacodynamic properties include enhanced platelet turnover, which decreases platelet exposure to aspirin, and genetic variants of COX, which would prevent its use as a substrate [11]. Accordingly, previous studies have demonstrated that patients with increased HAPR have increased risk of experiencing recurrent ischemic events.

In patients with stable CAD, calcium channel blockers (CCBs) are often co-administered with aspirin to treat hypertension and to alleviate angina pectoris. However, in patients with acute coronary syndrome, CCBs have not been shown to reduce the risk of initial or recurrent cardiovascular events [12–14]. It is not currently understood why CCBs, which are able to treat hypertension and promote coronary vasodilation, have not been shown to reduce the risk of coronary adverse events and/or mortality. We hypothesize that one of the mechanisms underlying their inability to do so relies on the pharmacological interaction between CCBs and aspirin (e.g., HAPR). In this study, we have examined the response to aspirin in patients with stable CAD who do or do not use CCBs to verify whether an adverse interaction exists between aspirin’s effectiveness and the usage of CCBs.

Methods

Study Population

This single-center observational study included an analysis of prospectively enrolled patients throughout January 2012 to June 2019 at one of the two campuses of the Rabin Medical Center, a tertiary hospital. Patients were either evaluated in the outpatient clinic (e.g., routine follow-up) or admitted to our hospital, for evaluation of angina symptoms. All patients had stable CAD at presentation and have undergone coronary angiography 3 months or more prior to recruitment, demonstrating significant CAD (defined as a stenosis of at least 50% in one or more epicardial coronary arteries by coronary angiography). All patients were regularly taking aspirin (75–100 mg qd) for at least 1 month prior to enrolment into the current study. Exclusion criteria were as follows: acute coronary syndrome at the time of platelet function testing, known malignant disease (patients with remission >2 years were eligible), sepsis or acute infection, active inflammatory/rheumatic disease, major surgery in the last 6 months (coronary artery bypass graft within the last 3 months), chronic liver failure, treatment with oral anticoagulation or anti-inflammatory medications (non-steroidal or corticosteroids), and allergy to or non-adherence with aspirin and thrombocytopenia (<100K/μL). The institutional review board approved the study, which was performed in accordance with ethical principles portrayed in the Helsinki Declaration.

Reagents and Devices

Following informed consent and patients’ verbal confirmation of adherence with aspirin within the last month including the day of the test, blood was drawn from the antecubital vein and sent to the platelet function test (VerifyNow). The VerifyNow system (Accumetrics, San Diego, CA) is a whole-blood assay based on light transmission measurements. The aspirin-specific cartridge, used in the current study, is designed and applied to assess platelet dysfunction caused by aspirin (the effect of aspirin on platelets). In the aspirin-specific assay, arachidonic acid activates platelets, and the activated platelets bind to fibrinogen-coated beads to form an aggregate. The degree of aggregation is quantified by a corresponding increase in light transmission and is reported in ARU. HAPR was defined as ARU >550 in consistency with previous studies [15, 16].

Data Sources and Classifications

Data were obtained from the hospital’s computerized medical records and included demographic and clinical characteristics, cardiovascular risk factors and comorbidities, procedural characteristics, and workup (e.g., blood tests, echocardiography). Glomerular filtration rate was calculated by the Modification of Diet in Renal Disease formula. Chronic kidney disease was defined as glomerular filtration rate below 50 mL/min/1.73 m². Anemia was defined as hemoglobin levels lower than 13.0 g/dL for men and 12.0 g/dL for women.

Statistical Analysis

Patient characteristics were presented as mean and standard deviation (SD) for continuous variables and percent for the categorical data. The chi-square or Fisher exact tests were used for analysis of categorical variables when appropriate. The unpaired student t-test or analysis of variance (ANOVA) was used for analysis of continuous variables. This was used to compare HAPR according to treatment with CCBs or not and the rates of HAPR according to each type of CCBs. A multivariate analysis study was performed using binary hierarchical logistic regression, to assess the independent factors associated with HAPR. Finally, we compiled a cohort of propensity score matched patients with a 1:1 ratio between CCB and control patients. The propensity score was derived from a multivariate logistic regression.
model that included treatment with CCB, considered to be the independent (outcome) variable, and all baseline clinical characteristics and procedural characteristics as covariates. The propensity score matched cohort was analyzed for the main combined outcome. Statistical analysis was performed using IBM SPSS Statistics 27 software. For each test, \( p < 0.05 \) was considered statistically significant.

**Results**

Overall, 503 patients were included in this study. There were 88 patients who had received CCBs—amlodipine (\( n=41 \)), lercanidipine (\( n=33 \)), nifedipine (\( n=8 \)), and diltiazem (\( n=6 \)). Mean age was 67.9±9.7 years for those treated with CCBs versus 66.5±11.4 years for the control group (\( p=0.288 \)), 77.3% were male vs. 82.9% (\( p=0.214 \)), and 34.7 vs. 36.9% had diabetes mellitus (\( p=0.121 \)). Differences were observed in the rates of past history of hypertension (83.9% in the CCB group vs. 63.5% in the control group, \( p<0.01 \)), rates of past MI (47.1% vs. 59.7%, \( p=0.031 \)), and past PCI (52.9% vs. 64.0%, \( p=0.039 \)). With respect to P2Y12 inhibitor use, 28.2% of patients in the CCB group were on clopidogrel vs. 26.9% (\( p=0.448 \)) in the control. 3.4% in the CCB group were taking ticagrelor versus 3.6% in the control group (\( p=0.945 \)). Prasugrel was not used in our study. The severity of atherosclerotic burden was assessed between groups. Mean Gensini score was 44.5±14.2 for those treated with CCBs versus 42.7±13.8 (\( p=0.271 \)) in the control. Additionally, rates of multivessel disease (MVD) were not statistically significant between groups, with 52.3% of patients in the CCB group having MVD versus 42.4% in the control (\( p=0.082 \), Table 1).

In patients taking CCBs, HAPR was observed in 15.9% in the CCB group and 7.0% in the control group (\( p=0.006 \), Table 2, Figure 1). Similarly, the mean ARU was 465.4±70.0 in the CCB group, compared to 445.2±60.0 for the control group (\( p=0.006 \), Figure 2). There were 7 patients with HAPR who were treated with amlodipine (17.1%), 5 patients with lercanidipine (15.2%), 1 with nifedipine (12.5%), and

**Table 1** Baseline characteristics of patients in the CCB and control groups

| Characteristic       | CCB group (n=88) | Controls (n=415) | \( p \)-value |
|----------------------|------------------|-----------------|--------------|
| Age (years)          | 67.9±9.7         | 66.5±11.4       | .288         |
| Male sex (%)         | 77.3             | 82.9            | .214         |
| BMI (kg/m²)          | 28.5±3.5         | 28.0±12.0       | .732         |
| CKD (%)              | 23.5             | 23.5            | .231         |
| Diabetes mellitus type 2 (%) | 34.7       | 36.9            | .121         |
| Hypertension (%)     | 83.9             | 63.5            | .000         |
| Hyperlipidemia (%)   | 85.1             | 83.0            | .642         |
| Current smoker (%)   | 38.0             | 35.1            | .786         |
| Family history (%)   | 54.8             | 56.4            | .787         |
| Prior MI (%)         | 47.1             | 59.7            | .031         |
| Prior CABG (%)       | 34.9             | 34.5            | .933         |
| Prior PCI (%)        | 52.9             | 64.0            | .039         |
| 1-Vessel disease (%) | 47.7             | 57.6            | .082         |
| 2-Vessel disease (%) | 37.5             | 32.3            | .082         |
| 3-Vessel disease (%) | 14.8             | 10.1            | .747         |
| Gensini score        | 44.5±14.2        | 42.7±13.8       | .271         |
| Statins (%)          | 89.5             | 90.7            | .747         |
| Beta blockers (%)    | 88.4             | 72.6            | .097         |
| ACE inhibitors (%)   | 72.6             | 62.0            | .065         |
| Clopidogrel (%)      | 31.6             | 26.9            | .448         |
| Ticagrelor (%)       | 3.4              | 3.6             | .945         |

CCB calcium channel blockers, BMI body mass index, CKD chronic kidney disease, CABG coronary artery bypass graft, PCI percutaneous coronary intervention, MVD multi-vessel disease, ACE angiotensin converting enzymes
In a binary logistic regression model, adjusted for sex, age, BMI, hypertension, hyperlipidemia, chronic kidney disease, diabetes mellitus type 2, smoking history, past myocardial infarction, and type of CCB, patients taking CCBs were 1.72 times more likely to have HAPR compared to those not taking CCBs (95% CI of 1.04–8.91, \( p = 0.047 \)).

A second factor associated with increased risk of HAPR was diabetes mellitus (OR = 2.21, 95% CI 1.26–4.34, \( p = 0.038 \)) (Table 3). The type of CCB (amlodipine, lercanidine, nifedipine, or diltiazem) showed no clear correlation with HAPR (\( p = 0.427 \)). There were also no differences in rates of HAPR when classified according to dihydropyridine or non-dihydropyridine, nor according to P-gp-inhibiting or non-P-gp-inhibiting CCBs. The propensity match score was able to form 79 matched pairs of CCB/control patients, showing similar results; patients taking CCBs demonstrated higher rates of HAPR (15.5% vs. 8.2%, \( p < 0.001 \)) and mean ARU (463.2±68.2 vs. 447.7±62.7, \( p = 0.02 \)). Following logistic regression of the matched groups, CCBs remained an independent predictor of HAPR (OR 1.56; CI: 1.22–1.93; \( p < 0.001 \)).

**Discussion**

The results of this study demonstrate a significant association between treatment of CCBs and aspirin responsiveness for patients with stable CAD. In these patients, the concomitant use of CCBs had a weakening effect on the pharmacologic potency of aspirin. Our study did not find an association between the type of CCB used and HAPR.

Our results are consistent with similar studies performed by Gremmel et al. which demonstrated that CCBs are associated with a decreased platelet response to clopidogrel [17, 18]. In these studies, tests were performed via flow cytometry and the VerifyNow system to conclude that the addition of CCBs to clopidogrel leads to a reduced antiplatelet response compared to clopidogrel alone. Potential mechanisms include the inhibition of cytochrome 3A4 and the inhibition of P-glycoprotein (P-gp) by CCBs [17, 18]. The first mechanism is based on clopidogrel as a prodrug that requires the activity of cytochrome 3A4 for its active form. If cytochrome 3A4 is inhibited by CCBs, clopidogrel cannot be appropriately metabolized, and its effect is diminished [17–21]. The latter mechanism considers that several CCBs inhibit the effect of P-gp, which is a necessary protein for clopidogrel’s intestinal absorption [18, 22]. One P2Y12 inhibitor that is not significantly reliant on P-gp is ticagrelor. Ticagrelor is unique in that it shows a high permeability through the Caco-2 monolayer, suggesting that it has higher efflux than influx potential through P-gp and does therefore
not rely on the enzyme for influx [23]. Whether or not CCBs affect the intestinal absorption of ticagrelor has not been studied yet. However, it is important to acknowledge that ticagrelor is metabolized to its more active form by C3A4, similar to clopidogrel [23]. It is possible that in line with clopidogrel, the active metabolite does rely on P-gp for absorption.

Our hypothesis is based on similar explanations for how CCBs attenuate aspirin’s effect, as evidenced by the increased rate of HAPR. The P-gp theory could be one of the mechanisms that accounts for our results. While some studies have concluded that aspirin is not a substrate for P-gp [24], others have found that P-gp causes efflux of aspirin [25] and is therefore associated with P-gp-based absorption in the gut. Hence, the concomitant use of CCBs with aspirin may lead to decreased bioavailability of the anti-thrombotic drug. In our study, the P-gp-inhibiting CCBs that were used included nifedipine and diltiazem. The association we found with increased HAPR was with use of CCBs overall, irrespective of the type of CCB; we estimate therefore that the P-gp theory accounts for only part of this association.

An additional protein that is strongly associated with aspirin resistance and shares similar efflux mechanisms with P-gp is the Multidrug Resistance Protein 4 (MRP4). MRP4 expression on platelets leads to efflux of cAMP and cGMP from the cytoplasm, leading to decreased activation of PKA and PKG. With reduced enzymatic activation, less calcium is mobilized from intracellular stores, leading to decreased actin cytoskeletal reorganization and platelet degranulation, necessary for platelet activation [26–28]. While there is no current study demonstrating the impact of CCBs on MRP4 expression, we believe that the use of CCBs may compound the effect that MRP4 has on HAPR due to further limitations on platelet-dependent calcium usage.

Another mechanism that could explain the increased HAPR with usage of CCBs is based on the Etingen study that demonstrated how CCBs enhance cholesterol ester (CE) hydrolysis [29]. The liver is the site for both CE and aspirin hydrolysis, with CE hydrolyzed into cholesterol and aspirin hydrolyzed into salicylic acid [30, 31]. When aspirin is hydrolyzed into salicylic acid, its antiplatelet activity is transformed to anti-inflammatory activity [32]. It is therefore also possible that CCBs upregulate the hydrolysis of aspirin in a similar fashion to the hydrolysis of cholesterol esters. The increased formation of salicylic acid depletes the aspirin load and leads to an increase in HAPR.

Finally, one of the mechanisms of action of aspirin is through the inhibition of prostaglandin (PG) H2 synthase (cyclooxygenase), preventing the generation of prostaglandin G2 and prostaglandin H2, and ultimately TXA2 [4]. For these events to occur, PGH2 synthase requires arachidonic acid liberation from phospholipids. However, arachidonic acid is released from phospholipids only once calcium levels rise within the cell. If calcium levels fail to rise due to the presence of calcium-blocking agents, aspirin may have a diminished ability to exert its anti-platelet effects [4, 7, 33]. In this scenario, CCBs hamper the antiplatelet effects of aspirin by limiting its potential to prevent TXA2 formation. It is therefore presumable that dihydropyridine and non-dihydropyridine should share similar effectiveness on HAPR due to arachidonic acid presence in both smooth and cardiac muscle [34]. Dihydropyridines primarily act on smooth muscle, whereas non-dihydropyridines primarily act on cardiac muscle; their influences would each cause an independent decrease in TXA2 formation.

In our study, dihydropyridine (amlodipine, lercanidipine, and nifedipine) had the same impact on HAPR as non-dihydropyridine CCBs (diltiazem). However, the number of patients receiving non-dihydropyridine CCBs was low (n=6). Therefore, any conclusions on the differential effects of dihydropyridine vs. non-dihydropyridine CCBs remain to be validated in larger studies in the future. Additionally, both P-gp-inhibiting (nifedipine and diltiazem) and non-P-gp-inhibiting CCBs (amlodipine and lercanidipine) were used with no identifiable differences in HAPR. Future studies, which compare larger number of patients receiving CCBs, may re-assess such associations between the type of CCBs and aspirin response.

While observational, our results are the first to examine the association between CCBs and HAPR and may suggest a mechanism for the lack of prognostic benefit in patients with ischemic heart disease treated with CCBs [12–14]. Understanding this mechanism may allow for a more personalized assessment of how to maximize cardio-protection for patients with cardiovascular risk factors. Further research is needed in order to determine the translational impact of this observation and to guide the medical management of patients with stable CAD treated with antiplatelet therapy.

**Limitations**

First, this is an observational study, meaning the generalizability of its findings are limited. Correlations can be used on the population observed, but causality cannot be concluded. Second, there was a relatively small number of patients who were taking CCBs, which again limits the generalizability of such a study, and in particular the differential effects of dihydropyridine versus non-dihydropyridine and P-gp-inhibiting versus non-P-gp-inhibiting CCBs. Third, platelet aggregation was used to measure efficacy of antithrombotic therapy. This was used as an alternative to the gold standard, which is thrombus formation in whole blood in the Badimon chamber [35]. Fourth, clinical outcomes were not measured in this study, which are necessary to understand what the aforementioned observations related to HAPR entail in terms
of adverse cardiovascular clinical outcomes. Specifically, a study assessing the impact of CCB-induced HAPR on the risk of thrombotic events is warranted. Nevertheless, as mentioned previously, other studies have concluded that an increased rate of HAPR confers a worse prognosis. Lastly, this is a retrospective study, which can lead to hindsight bias.

Conclusions

The current study reports a significant association between an increase in aspirin resistance, measured by both mean ARU and the rates of HAPR, and the use of CCBs. These findings provide a better understanding of the pharmacologic interaction between CCBs and aspirin in patients with stable CAD.

Author Contribution All authors contributed to the study conception and design. Material preparation and data collection were performed by Afek Kodesh, Eli Lev, Alejandro Solodky, and Leor Perl. Data analysis was performed by Leor Perl. Figures were made by Leor Perl. The first draft of the manuscript was written by Afek Kodesh and Leor Perl, and all authors read and edited the manuscript. All authors read and approved the final manuscript.

Data Availability Original research data will be available upon request.

Declarations

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Competing Interests The authors declare no competing interests.

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