British Journal of Medicine & Medical Research
4(29): 4770-4779, 2014

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Sensitivity to Epinephrine Determines Platelet Hyperreactivity in Myocardial Infarction under Antiplatelet Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KG, EB and OS designed the study, wrote the protocol and managed the literature search. Authors KG and AM assessed platelet aggregation. Authors AG and YH evaluated clinical and laboratory data. Authors KG and OS performed statistical analysis and its interpretation and made a draft of the manuscript, where after authors EB and AG critically revised it. All authors read and approved the final manuscript

Original Research Article

Received 2nd April 2014
Accepted 15th May 2014
Published 27th June 2014

ABSTRACT

Aim: To investigate the role of platelet adrenoreactivity in development of hyperreactive platelet phenotype in patients with myocardial infarction receiving antiplatelet therapy.
Study Design: Cohort prospective study.
Place and Duration of Study: Histology Department of Donetsk National medical university; Institute of Emergent and Rehabilitation Medicine, Donetsk, Ukraine, between June 2011 and September 2013.
Methodology: The study was carried out on 36 patients with acute ST-elevation MI receiving double antiplatelet treatment. After assessment of epinephrine (5 μM) induced aggregation two groups of patients were identified: with high (1st group, with aggregation more than 40%) and low (2nd group, aggregation less than 40%) adrenoreactivity. Within both groups platelets' reaction to the main agents (ADP (5 mkM), collagen (5mkg/ml), ristocetin (0.5mg/ml); thrombin (1U/ml)), was measured. Since a mixture of agonists in low concentration is present at the site of coronary arteries occlusion, subthreshold concentrations of agonists were used in addition to EC50 in order to analyze their

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potentiating effect on aggregation. 10 healthy volunteers were taken as a control group. The results were analyzed using MedCalc software.
Results: 75% (27) of patients with MI on double antiplatelet therapy developed hyperreactivity to epinephrine. Comparison of platelet reactivity among the patients with high and low adrenoreactivity showed that hypere adrenoreactivity phenomenon was associated with higher platelet response to ADP, as well as to adhesive agents (ristocetin and collagen). Whereas subthreshold concentration did not exhibit much potentiating effect among the patients with low initial adrenoreactivity, hyper adrenoreactive patients exhibited rather powerful potentiation of effects of ADP and epinephrine.
Conclusion: Adrenoreactivity plays an important role in the responsiveness to different agonists and could be a mechanism of tolerance/resistance to antiplatelet therapy among the patients with MI.

Keywords: Platelets; adrenoreactivity; myocardial infarction; epinephrine; aggregation; antiplatelet therapy.

1. INTRODUCTION

Acute MI is initiated when an atherosclerotic plaque ruptures, exposing subendothelial molecules, notably collagen and von Willebrand factor (vWF), to circulating platelets. It is through the signaling cascade from receptors to vWF and collagen on their surface (GPIb-V-IX GP and VI correspondingly) that platelets become activated, adhere to the zone of lesion, synthesize thromboxane A2 (TxA2) and degranulate, releasing content of their granules (ADP, PAF, serotonin etc) and involving other platelets into the growing thrombus [1,2]. The final step of platelet activation is assembling integrin IIbIIIa at the surface of platelets, which makes it apt to form fibrinogen bridges with other activated platelets. What is more, platelets establish an important link between thrombogenesis and coagulation cascade due to several factors. Firstly, their granules contain coagulation factors (platelet factor 4), contributing to the so-called intrinsic coagulation cascade, resulting in thrombin generation at the site of rupture; the latter is not only the central protease in coagulation, responsible for fibrinogen cleavage with subsequent fibrin clot formation, but is a potent platelet agonist acting through PAR1 and PAR 4, exacerbating platelet plug growth. In addition, phospholipids asymmetry of the platelet membrane serve as a platform for coagulation cascade, further contributing to plug formation. As a result, at the site of a ruptured plaque, a thrombus grows, blocking blood supply to the myocardium involved [2,3].

The “golden standard” of myocardial infarction (MI) therapy includes administration of aspirin, that diminishes generation of TxA2, and clopidogrel, that brings down ADP effect [4]. Despite a significant success of such therapy in a large number of patients, a subgroup of patients remains with preserved high platelets reactivity to different stimuli. Clinically defined as a resistance to antiplatelet therapy, the problem of hyperreactive platelets is a heterogenous condition that might be explained by gene polymorphism of receptors or signaling molecules, inadequate drug modification of clopidogrel (by CYP enzymes), as well as persistent activation of a signaling molecule involved in platelet response [5].

It was shown that platelet hyperreactivity is associated with activated GPIIb/IIIa integrin receptor and P-selectin exposition, activated factor (FVa) and annexin V expression, as well as with increased procoagulant activity of platelets due to platelet-specific secreted proteins

like platelet factor 4 (PF4) etc. As cardiovascular pathology is associated with dyslipidemia and oxidative stress, one of the possible mechanisms that increase the risk of thrombotic complications is oxidation of choline glycerophospholipids that promote platelet activation and hyper reactivity [6,7].

In addition, an important role in development of hyperreactive phenotype of platelets might be played by a certain "cocktail" of platelets' activators that exist at the site of ischemic myocardium and in plasma in physiological concentrations, much lower than that of agonists used *In vitro*. Studies on synergistic action of platelets showed that they are likely to act in concert, potentiating each other, exhibiting a potent aggregation and, presumably, participating in the development of platelet hyper reactivity phenomenon [8]. Since MI is not only accompanied by massive accumulation of purines (ADP, ATP) around oxygen-depleted myocardium, but is connected to a potent activation of sympathoadrenal system, leading to overproduction of catecholamines into blood, it was reasonable to evaluate possible synergism that these agonists exhibit. Despite the fact that epinephrine has only a moderate effect on platelets as it acts through a limited number of α2-adrenoreceptors present on platelet membrane, it has a common signaling pathway with a P2Y12 receptor to ADP that is blocked by clopidogrel (Gi –pathway) [9]. Thus, in the stressful condition of the acute coronary syndrome epinephrine may restore platelets reactivity by mimicking action of ADP on P2Y12 receptor. Consequently, platelets adrenoreactivity may influence overall platelets phenotype following therapy administration. Aim of the research was to investigate the role of platelet adrenoreactivity in development of hyperreactive platelet phenotype in patients with myocardial infarction receiving antiplatelet therapy.

2. MATERIALS AND METHODS

2.1 Study Design

A cohort observational study was conducted on patients with MI enrolled into Institute of Emergent and Rehabilitation Medicine (Donetsk, Ukraine) between June 2011 and September 2013.

2.2 Study Population

The study was carried out on 36 patients with acute ST-elevation MI. All patients received treatment according to the ESC Guidelines for the management of acute myocardial infarction; it included double antiplatelet treatment (clopidogrel 75mg daily following the loading dose of 300mg and aspirin 75-100mg daily following the loading dose of 300mg), beta-blocker, statin, aldosterone antagonists, angiotensin-converting enzyme inhibitor, nitrates. Patients underwent percutaneous coronary intervention accompanied by anticoagulant administration (heparin).

Inclusion criteria were age more than 35 years, ST-elevation MI documented by ECG, admission to hospital during working week (monday to friday) no later than 24 hours after first symptoms of infarction. Patients with ECG signs of ST-elevation MI were admitted to the coronary care unit of the emergency department. Exclusion criteria were the following: any documented malignancies, hemostasis disorders, cardiogenic shock at the time of admission, full left His bundle block. Several variables were identified that could have influenced the results: age, sex, arterial hypertension, heart failure, MI and stroke in anamnesis.

2.3 Study Procedure

Blood sampling took place at the site of admission, at Institute of Emergent and Rehabilitation Medicine, Donetsk, Ukraine. Blood was collected from the antecubital vein into plastic syringes containing sodium citrate at a final concentration of 0.38% with proportion 9:1 and centrifuged at 200×g for 20 minutes at 25ºC to prepare platelet-rich plasma. Platelet aggregation was evaluated 24 hours after beginning of therapy.

2.3.1 Platelet aggregation to agonists in EC50

Born method aggregometry (aggregometer Chrono-Log, USA) was used to measure platelet aggregation to the range of agonists in EC50 following stimulation with ADP (5mkM), epinephrine (5mkM), collagen (5mkg/ml), ristocetin (0.5mg/ml) and thrombin (1U/ml) (Sigma).

After dividing patients into two groups with high and low adrenoreactivity (response more/less than 40%), and evaluating their clinical characteristics, they were compared on the basis of their response to the range of agonists.

2.3.2 Platelet aggregation to agonists in subthreshold concentrations

Additionally, platelet responses to agonists in subthreshold concentration was analyzed (ADP-0.5mkM, epinephrine-0.1mkM) as separately, as in combination. Subthreshold concentration was defined as a concentration that exhibited no more than 15% aggregation studied in control group. Pairs of agonists were added at the platelet rich plasma simultaneously, immediately before the beginning of registration.

2.3.3 Statistical analysis

MedCalc version 12.3 (MedCalc Software Inc, Broekstraat, Belgium) was used for statistical analysis. Descriptive statistics were used to analyze and report the data. For presentation of nominal data, the percentage and standard error were calculated; for presentation of numeric data, the median and standard error were calculated. The chi-squared and the rank Kruskal-Wallis and Dunn's tests were used to determine differences between patients of two groups (10). To evaluate reliability of the study, statistical power was calculated: for comparison of ADP effect in two groups (EC50), it reached 97%, whereas for agonists in subthreshold concentration it was 48% (two- tailed Alpha 0.05).

3. RESULTS AND DISCUSSION

3.1 Platelet Aggregation in Response to Agonists in EC50

At the first day after beginning of antiplatelet therapy overall platelet aggregation among all patients enrolled into study was the following: ADP: 50±6.82 (30-68), epinephrine: 66±7.54 (50-79), collagen: 80±10.74 (30-100), thrombin: 23±10.33 (10-70); the range of aggregation to most agonists differed significantly (1-100%). Among 53% of patients, high response to ADP persevered despite treatment. Aggregation parameters were not associated neither with demographics, nor with severity of atherothrombosis (coronography data). Correlation analysis did not reveal any relations of platelet response to different agonists. Consequently, we suggested that effectiveness of treatment depends on parameters of individual reactivity,

namely, adrenoreactivity, bearing in mind the role of catecholamines in pathology of cardivasculary system. To check this hypothesis, platelet response to different agonists was compared among patients with different adrenoreactivity.

According to the distribution plot of patients with different response to epinephrine, two groups were identified: those with response more and less than 40% (Fig. 1). Two groups were comparable in terms of age, gender, heart failure class, arterial hypertension anamnesis, leukocyte count (Table 1).

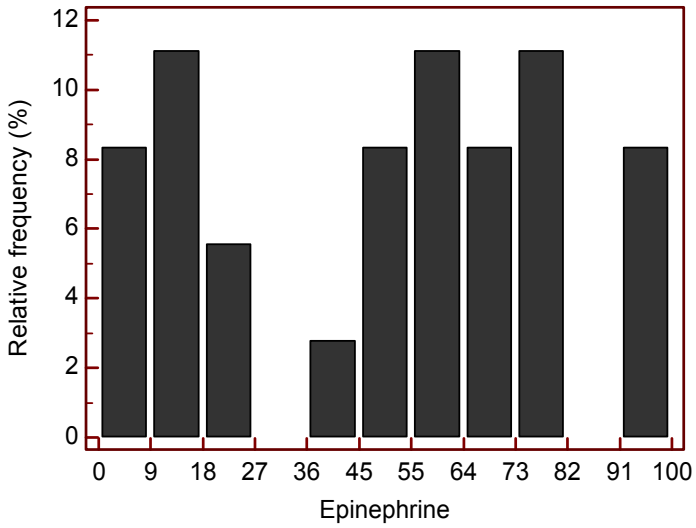

Fig. 1. Relative frequency of patients according to platelet response to epinephrine
A bimodal distribution of patients according to platelets' response to epinephrine suggests existence of two subgroups of patients, with high (≥40%) and low (<40%) response to epinephrine

Table 1. Comparative characteristics of two groups of patients, divided on the basis of platelet response to epinephrine

Characteristic	1 group (epinephrine≥50%)		2 group (epinephrine<50%)	
	Absolute number	%	Absolute number	%
Overall patients	22	61	14	39
Male	21	95	10	71 (*P*=.147)
Female	1	5	4	29
Age, years	58.7±1.9		56.4±3.26	
Stroke in anamnesis	1	0.04	0	0
Hypertension in anamnesis	15	68	5	36 (*P*=.299)
Congestive heart failure, class III-IV by NYHA	6	27	2	14 (*P*=.84)
Leukocytes count,×10^9/L	12,42±0,68		13,92±1,14	

As the table shows, two groups were comparable in terms of demographic, clinical and laboratory data. They differed significantly in their response to ADP (Fig. 2), as well as to all

studied agonists (Table 2). Furthermore, according to ANOVA test, adrenoreactivity influenced response to ADP (F=4.76, *P*<.01), collagen (*P*<.01) and ristocetin (*P*<.01), but did not influence response to thrombin (*P*=.94).

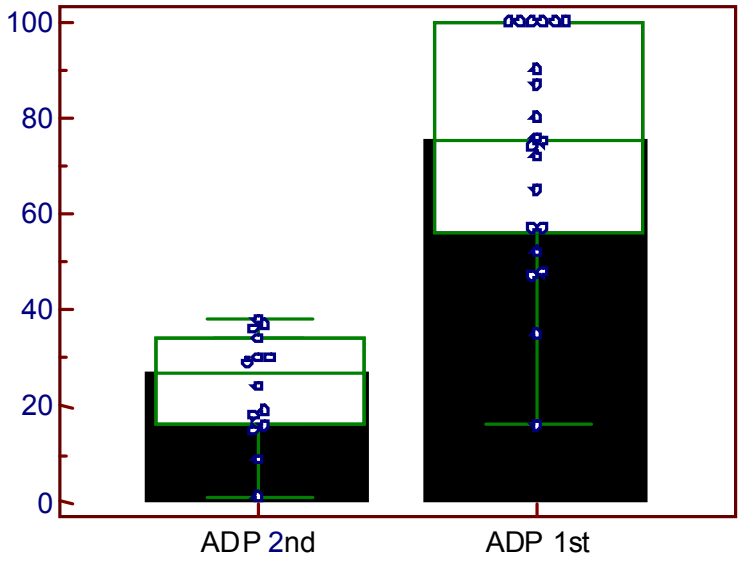

Fig. 2. Platelets response to ADP in patients with high (1 group) and low (2 group) adrenoreactivity after one day of antiplatelet therapy

Hypereadrenoreactivity was associated with high platelet response to ADP. Power of study 97% (two-tailed Alpha 0.05)

Table 2. Platelet response to the range of agonists among patients with different adrenoreactivity

Agonist	1 group Me±m (CI)	2 group Me±m (CI)	*P*
Epinephrine	78±5.832(61-100)	10±4.8(5-26)	<.001
ADP	68±7.846(40-87)	25±7.794(15-40)	.007
Collagen	71±8.2(53.5-89.4)	47±4.7(37-57)	.018
Ristocetin	82±6.7(67.9-97.2)	56±4.9(45-65)	.008
Thrombin	48±16.8(8-100)	9±9(5-21)	.038

3.2 Platelet Response to the Pairs of Agonists in Subthreshold Concentration

Patients were evaluated on the basis of their response to agonists in subthreshold concentration as those are the concentrations present in the vicinity of a plugged vessel; consequently, this is a mixture of such agonists that might induce a full platelet response and exacerbate plug growth. Predictably, a single agonist in a subthreshold concentration (ADP, epinephrine) did not induce a notable response: 10±2.22% (5-15) and 10±2.6% (3-16) correspondingly; when used together, however, ADP and epinephrine developed a potent irreversible aggregation with a degranulation phase in patients with high response to epinephrine: 36±8.7% vs 17.5±4.41% (*P*=.037). Consequently, high adrenoreactivity was accompanied by a higher response to ADP and epinephrine in combination (Figs. 3, 4).

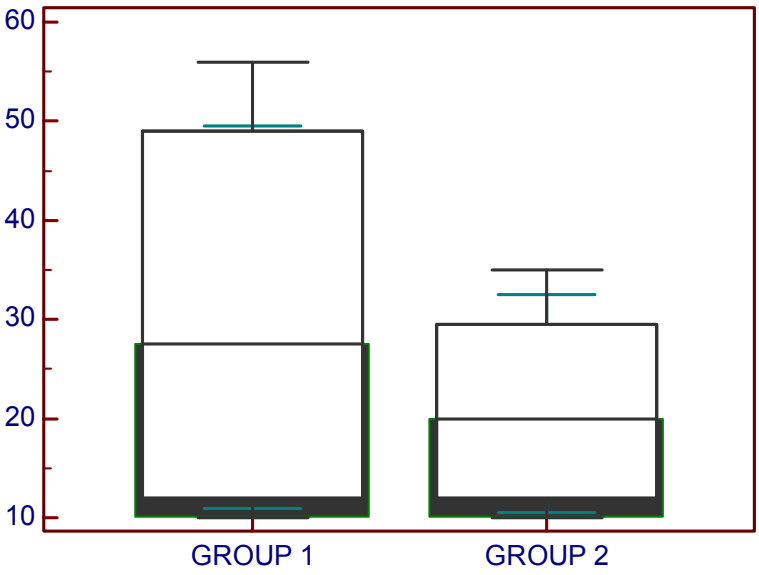

Fig. 3. Platelet response to a mixture of epinephrine and ADP in subthreshold concentration in patients with high (1st group) and low (2nd group) adrenoreactivity
Power 48% (two-tailed Alpha 0.05)

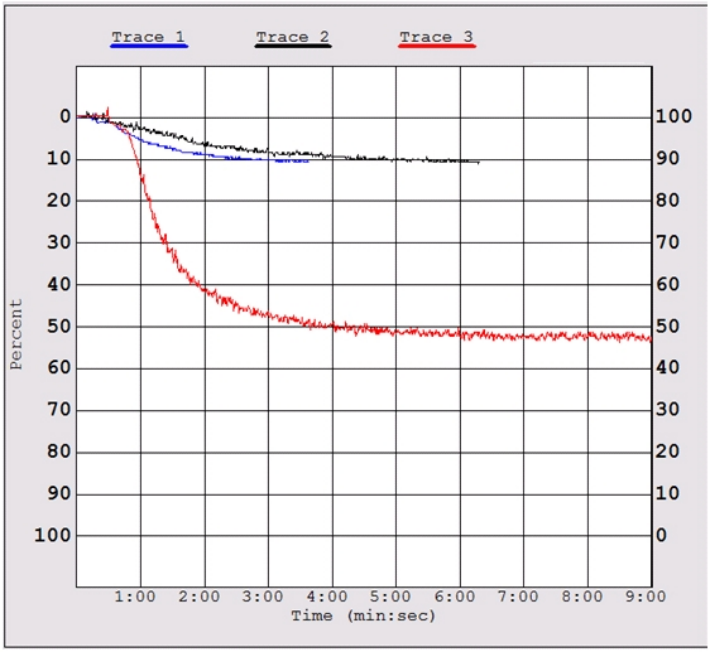

Fig. 4. Potentiating effect of epinephrine on ADP in a patient with high adrenoreactivity (1st group)
Trace 1 corresponds to epinephrine, Trace 2–ADP, Trace 3–incubation of platelets with ADP and Epinephrine

3.3 Discussion

Growing body of evidence shows that antiplatelet treatment improves short-and long-term prognosis in patients with coronary artery disease [3]. Blockage of platelet activation by aspirin and clopidogrel among the patients with MI prevents coronary arteries rethrombosis. Hyperreactive platelet phenotype is known to be associated with development of acute coronary syndrome and secondary complications after MI [1,4,5]. Despite differences in methods of detection, high response to different stimuli following administration of antiplatelet agents clinically implies resistance to therapy.

Our study provides three major findings. Firstly, more than half of all MI patients, receiving antiplatelet therapy, developed high platelet reaction to epinephrine despite antiplatelet treatment. Furthermore, hyperadrenoreactivity was associated with high response to ADP, which implies resistance to standard therapy [5]. The underlying mechanism might include persistent activation of a signaling molecule common for both pathways; among the possible "players" might be IP3K, activated through α2–adrenoreceptors or P2Y12 receptors as they both act through Gi-protein [3,9].

Secondly, apart from preserved ADP response, patients with high adrenoreactivity developed higher response to collagen and ristocetin, whose effects are realized through GPI and GPVI receptors [1,9]. These glycoproteins not only represent mechanisms of platelet adhesion to the endothelium, but also, through Src-kinase pathway, stimulate phospholipase A2, that initiates arachidonic acid metabolism resulting in production of a potent platelet activator thromboxane A2 [2,3].

From that perspective, preserved high platelet response to the range of agonists despite intake of aspirin and P2Y12 inhibitors can suggest either "escape" of platelets from aspirin and clopidogrel action, or involvement of other agonists such as serotonin, angiotensin II and thrombin into platelet response. In addition, impairment of negative regulation of thrombogenesis might be responsible for hypereactivity, which is connected to the inhibition of adenilatcyclase and cAMP production following Gi protein activation [3,9]. Such diverse molecular interactions following activation of adrenoreceptors, purinoreceptors and PAR might not only explain their synergism in development of platelet response, but underlie platelet hyperreactive platelet phenotype, leading to occlusion of coronary arteries [11]. Studies by Béres BJ et al. [12] held on patients with stable angina receiving standard double antiplatelet therapy (75mg clopidogrel and 100 mg of aspirin per day) might be the proof of such hypothesis. They showed that low dose of epinephrine (10-9g/ml) considerably increased platelet aggregation caused by ADP (up to 43% compared to initial 26.5%) and collagen (42% compared to 17%; $P<.001$). Adding selective α2A blocker atipamezole decreased, but did not fully abolished adrenaline action.

Test with subthreshold concentration of agonists allowed us to establish the third finding. While low doses of epinephrine and ADP used solely caused only low platelet response (their action did not differ from control), they exhibited summation of effect while used together in the group with low adrenoreactivity, reaching 30% of aggregation, and, furthermore, caused potentiating effect among the hyperreactive group of patients, reaching up to 50% of aggregation, which emphasizes importance of signaling model of platelet hyperreactivity and leads to the suggestion that evaluation of platelet response to a combination of ADP and epinephrine in low doses might be highly informative for assessment of antiplatelet therapy effectiveness.

4. CONCLUSION

Adrenoreactivity plays an important role in the platelet responsiveness to different agonists and tolerance/resistance to antiplatelet therapy among the patients with myocardial.

5. LIMITATIONS OF THE STUDY

Due to small number of studied population, its heterogeneity due to comorbidity and medication prescribed, the study might be considered a hypothesis-generating and should lead to a further larger-scale prospective investigation on a homogenous population.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of the material and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that the study design have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=581&id=12&aid=5082