Evaluation of the Anti Platelet Aggregation Effects of Diltiazem

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Submission: October 06, 2016; **Published:** November 03, 2016

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Abstract

Background: Diltiazem belongs to Ca++ channel blockers group of drugs used for multiple disorders like hypertension, cardiac arrhythmias, angina pectoris and peripheral vascular disease. It causes relaxation of the vascular smooth muscles by a well known mechanism that is blockage of L-type of the calcium channels inhibiting the entry of Ca++ into the muscle cells. Current study explored its effects on platelet aggregation and their molecular basis.

Methodology: This experimental study was conducted at Dr. Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi. Blood samples from 20 normal volunteers were taken under aseptic measures after informed consent. Blood was centrifuged at 1000RPM to receive PRP (platelet rich plasma). PRP was mixed with the PAF and Arachidonic Acid with the help of Eppendorf pipette and placed in aggregometer to observe the aggregation at different concentrations. Then different concentrations of Diltiazem were added to these solutions to observe the inhibition of platelet aggregation at different concentration. IC50 of Diltiazem was calculated by dose response curves.

Results: We observed that Diltiazem successfully reduced the PAF and AA induced platelet aggregation at IC50 =15uM (Inhibitory Concentration). That is an additional aspect of this agent.

Conclusion: Diltiazem has dual role in the treatment and prevention of ischemic heart disease and ischemic stroke by reducing the blood pressure and inhibiting the platelet aggregation.

Keywords: Diltiazem; Arachidonic acid; PAF; PRP; RPM; Platelet aggregation

Abbreviations: MI: Myocardial Infarction; STEMI: ST Elevation Myocardial Infarction; PAF: Platelet Activating Factor; AA: Arachidonic Acid; PRP: Platelet Rich Plasma; RPM: Rounds Per Minute; PPCI: Primary Percutaneous Coronary Intervention; ROC: Receptor Operated Channels; TRPC: Transient Receptor Potential Channels

Introduction

Calcium channel blockers are the recommended treatment option in hypertensive patients with diabetes or angina [1]. Diltiazem is a benzothiazepine derivative agent affecting both cardiac and vascular smooth muscles. Its oral bioavailability is 40-65% with half-life of 3-4hours and minimum therapeutic plasma levels is from 50-200ng/ml. Many studies suggest that calcium channel blockers interfere with platelet aggregation in vitro and prevent the development of atheromatous lesions in animals [2]. Human circulating platelets serve many important functions in human body. Platelets are prime modulators of hemostasis [2]. They help in preventing bleeding by coagulation at the site of bleeding. Platelet aggregation is an important step in the coagulation process. Excessive aggregation of these platelets is the cause of many cardiovascular diseases like atherosclerosis, angina, myocardial infarction and ischemic stroke. Upon vascular injury, the primary adhesion of platelets with sub endothelial extracellular matrix is mediated by adhesive molecules under high shear stress to form a monolayer.

This is followed by subsequent recruitment of additional platelets from circulation by releasing stored dense granules to form a platelet plug [3]. Anti platelet drugs like Clopidogril and Aspirin are used to reduce the platelet aggregation for the prevention and treatment of cardiovascular diseases. Resistance to these traditional drugs is observed as recurrent attacks of MI and stroke are being observed despite the use of these medicines. The reported incidence of resistance to these drugs varies greatly, from less than 55 to 75% [4]. In STEMI patients, in particular after primary percutaneous coronary intervention (PPCI), high residual platelet reactivity is associated with increased risk of recurrent chances of coronary thrombosis despite of dual antiplatelet therapy [5]. So there is need of safe alternative agents that may reduce the platelet aggregation many researchers are working for this purpose. Platelet aggregation is reported to be increased in hypertension and diabetes. We studied the effects of Diltiazem, an old and well known Ca channel blocker on the platelet aggregation because many studies emphasized the role of calcium in the aggregation process.

Material and Methods

Chemicals

Diltiazem, PAF, A A were purchased from Sigma Chemical Co. (St. Louis, Mo. USA).

Sample collection

Blood samples were taken from twenty normal human volunteers by probability sampling after written and informed consent. Patients using any anti platelet drugs were excluded on history and individuals having bleeding disorders or low platelet count were also excluded. A sample 5cc blood was drawn by using 22guage canola from upper limb through venepuncture under aseptic measures. The age range of the participants was 18 to 40 years.

Preparation of platelet rich plasma

Blood was mixed with the Sodium citrate (3.8%) to obtain a 9:1 solution to avoid coagulation. Sample was centrifuged at 1500 RPM at 20oC for 15minutes to obtain PRP. Platelet count was calculated using contrast microscopy and aggregation studies were conducted at 37oC with platelet count ranging 2.5 to 3.0x108/ml of PRP [6].

Methodology

Dual channel Lumi-Agregometer Model 400 (Chronolog Corporation, Chicago, USA) was used to measure platelet aggregation. Aggregation was induced by adding 0.05 ml of PAF to 0.45ml of PRP making the final volume 0.5 ml firstly and then secondly by adding 0.05 ml of AA separately. Both PAF 0.05ml and AA0.05ml were mixed with 0.40ml of PRP to observe the synergistic aggregation thirdly. Finally the anti aggregatory effects of the Diltiazem were observed by pre treatment of PRP

with it (Diltiazem) for one minute followed by sub threshold concentrations of PAF and A A. Aggregation was recorded for 5 minutes as change in light transmission as a function of time. Dose response curves were constructed PAF and AA and to calculate the IC50 value of the Diltiazem using 5 different doses.
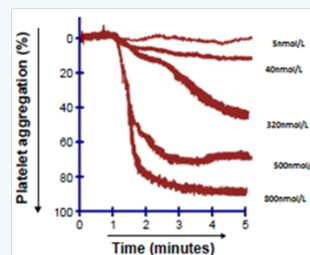
Results

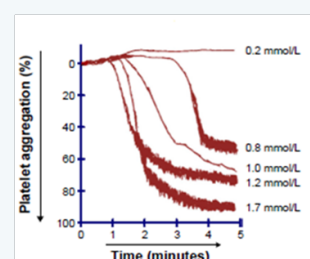**Figure 1:** PAF induced platelet aggregation.

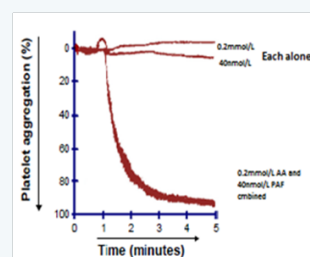**Figure 2:** Arachidonic Acid induced platelet aggregation.

Figure 3: Synergistic platelet aggregation between AA and PAF.
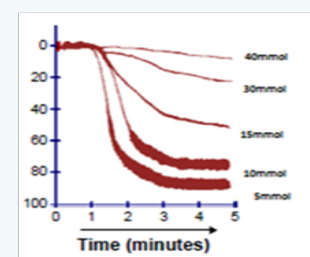

Figure 4: Inhibition of synergistic aggregation by Diltiazem.

The results showed that AA (0.2-1.73mmol/L) increased the platelet aggregation in a concentration dependent manner and the same was true for PAF (5-800 nmol/L) represented by (Figures 1 & 2) respectively. Synergism was observed when sub

threshold concentrations of AA (0.2mmol) and PAF (40nmol) were combined together (Figure 3) resembling the effects of higher concentrations of AA (1.73m mol) and PAF (800nmol). It was observed that Diltiazem inhibited the synergistic aggregation of platelets by AA and PAF at an IC50 of m mol shown by (Figure 4).

Discussion

Both AA and PAF are potent mediators of platelet aggregation. Platelet membrane contains receptors for PAF while AA exerts its effects through its metabolite thromboxane A2. Many studies suggest the central role of Ca++ in platelet aggregation process. Intra platelet concentration is increased through PLC and IP3 mediated release of Ca++ from endoplasmic reticulum, ROC (receptor operated channels) and TRPC (transient receptor potential channels) that mediate Ca++ entry through the plasma membrane [7]. Paul Erne [8] proved that intra sytosolic Ca++ concentration was increased in hypertensive patients in comparison to normotensives. Diltiazem a Calcium channel blocker drug reduced the platelet aggregation in a dose dependent manner proving its dual effects along with smooth muscle relaxation. Our results show similarity with the study of Rybak ME & Renzulli LA [9] who observed antiaggregatory effects of calcium channels blockers in ADP and collagen induced experiments. However they used multiple drugs and our study checked Diltiazem only against AA and PAF induced aggregation. Previously we studied the effects of Diltiazem on adrenaline and serotonin induced platelet aggregation and fond that it inhibited this aggregation at an IC50 of 40uM [10].

Studies on hypertensive patients using Amlodepine showed no significant alteration in platelet aggregation in whole blood and PRP between normal and experimental group [11]. Which is in contrast of our study and shows that probably there exists a difference between the Ca++ channel blockers that needs further exploration. Calcium channel blockers. Blache D et al. [12] proved that Ca++ and thrombin dependent release of serotonin was inhibited by Nitrendipine at 20microM. Studies by H Dai et al. [13] on Nefidipine along with aspirin also show accordance with our studies. All studies conducted in the world level on different types of calcium channels blockers proved that they have the anti platelet properties that is a very important aspect for these agents. Unfortunately no single study including the current study covered all aspects of platelet aggregation like all stimulants of aggregation needs to tested at the same time and second messengers as well. The receptor and channels evaluation still remains unresolved issue how these agents exactly inhibit platelet aggregation needs still further exploration. Apart from the molecular basis of inhibition of platelet aggregation these agents are providing an additional benefit to their users that are they reduce the chances of ischemic processes.

Conclusion

It is concluded from this study that Diltiazem has strong anti aggregatory effects on platelets in addition to its well known effects on smooth muscles relaxation.

Recommendation

Studies on blood from patients using Diltiazem are recommended to support and to confirm the results of our study. We also recommend studies involving Adrenaline, PAF, ADP, A A, Collagen, Thrombin, Serotonin and inhibitors of the second messengers all together for exploring the multi factorial process.

References

1. Kyle Melin (2015) Antihypertensives. In: Richard A Harvey, Lippincott Illustrated Reviews Pharmacology, (6th edn), Wloters Kluwer, China, pp. 225-239.

2. Bertram G Katzung (2012) Vasodilators and the treatment of angina pectoris. In: Bertram G Katzung, Susan B Masters and Anthony J Trevor (Eds.), Basic and Clinical Pharmacology, McGraw Hill Companies, USA, pp. 193-210.

3. Khan N, Farooq AD, Sadek B (2015) Investigation of cyclooxygenase and signaling pathways involved in human platelet aggregation mediated by synergistic interaction of various agonists. Drug Des Devel Ther 9: 3497-3506.

4. James L Zehnder (2012) Drugs Used in Disorders of Coagulation. In: Bertram G Katzung, Susan B Masters and Anthony J Trevor (Eds.), Basic and Clinical Pharmacology, McGraw Hill Companies, USA, pp. 601-617.

5. Martin Marinšek, Andreja Sinkovič (2016) Ramipril and Losartan Exert a Similar Long-Term Effect upon Markers of Heart Failure, Endogenous Fibrinolysis, and Platelet Aggregation in Survivors of ST-Elevation Myocardial Infarction: A Single Centre Randomized Trial. Bio Med Research International Volume.

6. Shah BH, Saeed SA (1995) Phosphatidyl inositol 3-Kinase inhibitor, Wortmannin inhibits 5-hydroxytryptamine-mediated potentiation of platelet aggregation induced by epinenephrine. Res Commun Mol Pathol Pharmacol 89(2): 157-164.

7. Varga Szabo D, Braun A, Nieswandt B (2009) Calcium signaling in platelets. J Thromb Haemost 7(7): 1057-1066.

8. Erne P, Bolli P, Bürgisser E, Bühler FR (1984) Correlation of platelet calcium with blood pressure-Effect of antihypertensive therapy. N Eng J Med 310(17): 1084-1088.

9. Rybak ME, Renzulli LA (1992) Effect of calcium channel blockers on platelet GPIIb-IIIa as a calcium channel in liposomes: comparison with effects on the intact platelet. Thromb Haemost 67(1): 131-136.

10. Saeed A, Motiwala A, Qureshi ZU, Khan R, Ali A, et al. (2006) Mechanisms for Platelet aggregation mediated by G-protein coupled receptors in

human platelets. JCPSP 16(2): 167.

11. C Rostagno, A Colella, E Chiarantini, D Prisco, G F Gensini (1995) Effects of Amlodipine on Platelet Aggregation in Hypertensive Patients. Clinical Drug Investigation 9(5): 255-259.

12. Blache D, Ciavatti M, Ojeda C (1987) The effects of Calcium channel blockers on blood platelet function specially on calcium uptake. Biochim Biophys Acta 923(3): 402-412.

13. H Dai, J Chen, Q Tao, J Zhu, F Zhang, et al. (2003) Effects of Diltiazem on platelet activation and cytosolic calcium during per cutaneous transluminal coronary angioplasty. Postgrad Med J 79: 522-526.