AN INVITRO COMPARITIVE STUDY OF ANTIPLATELET DRUG COMBINATIONS IN SWISS ALBINO MICE

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ABSTRACT
The present study determines the effect of two combinational antiplatelet drugs on thrombin generation in swiss albino mice by comparing with already existing single and combinational antiplatelet drugs. The thrombin generation was monitored invito-in recalcified-plasma of clotting by evaluating the parameters like bleeding time, clotting time, total platelet count, formed fibrin content, thrombin time and rate of thrombin generation with standard therapeutic doses. Tirofiban+Ticlopidine and Tirofiban+Dipyridamole combinational drug treatment gives significant results in delaying of thrombin generation than the Aspirin & Aspirin+ Clopidogrel. Delaying in the thrombin generation is useful incidence in patients, who are under the acute myocardial treatment and management. The present study revealed that the combinational antiplatelet drugs considerably delays the thrombin generation than single drug treatment.

Keywords: coronary artery disease, platelets, antiplatelet drugs, thrombin & fibrin.

INTRODUCTION
Various mechanisms, including endothelial cell nitric oxide and prostacyclin, promote blood fluidity by preventing platelet stasis and dilating intact blood vessels. These mediators are no longer produced when the vascular endothelium is disrupted. Under these conditions, platelets adhere to the damaged intima and form aggregates. Initial platelet adhesion is to Von Willebrand’s Factor (VWF), previously secreted by endothelial cells in to sub-endothelium. VWF binds to receptors on the platelet surface membrane (glycoprotein lb/Ix). Platelets anchored to the vessel wall undergo activation and release mediators from storage granules. Adenosine Di phosphate (ADP). other biochemical changes resulting from activation include hydrolosis of membrane phospholipids, inhibition of adenylate cyclase, mobilization of intracellular Ca++, and phosphorylation of intracellular proteins. Arachidonic acid is converted to thromboxane A₂ reversibly by many NSAIDs. ADP, thromboxane A₂, and other mediators draw additional platelets to the injured endothelium (platelet aggregation) and activate them. Another receptor is assembled on the platelet surface membrane from glycoprotein I Ib/ IIa. Fibrinogen binds to the glycoprotein II b / III complexes of adjacent platelets, connecting them [1, 2]. Platelets provide surfaces for the assembly and activation of coagulation complexes and the generation of thrombin. Thrombin converts fibrinogen to fibrin, fibrin strands bind aggregated platelets to help secure the platelet-fibrin hemostatic plug [3, 4, 5]. Fibrin deposition and lysis must be balanced to maintain and remodel the hemostatic seal during repair of an injured vessel wall. The fibrinolytic system dissolves fibrin by means of plasmin, a proteolytic enzyme. Fibrinolysis is activated by plasminogen activators released from vascular endothelial cells. Plasminogen activators and plasminogen from plasma bind to fibrin. Plasminogen activators catalyze cleavage of plasminogen, creating plasmin. Plasmin produces soluble fibrin degradation products that are swept in to the circulation [6].

At high flow, as in a wound or an artery, generated thrombin is easily washed away and its effects are limited to the area where no flow is possible, i.e. the interstices of the platelet aggregate. In venous thrombosis thrombin is not washed away, so that large clots form around the primary, platelet rich head of the thrombus. Defective secondary hemostasis in our opinion is due to fibrinolysis induced re-bleeding. Thrombin is required for the formation of thrombin induced fibrinolysis and Fibrinolysis is not sufficiently inhibited if not a sufficient amount of thrombin has been formed [7].

MATERIALS AND METHODS
Drugs
the drugs which were utilized in the present study were procured from the standard pharmaceutical industries of INDIA like Nicholas Piramal India Limited (NPIL), Mumbai and Zydus Cadila Health Care Limited, Ahmedabad. Aspirin (ASA)-75mg tab, Clopidogrel (NOKLOT)-75mg tab, Ticlopidine(TICLOP) 100 mg tab& Dipyridamole(PERSANTIN) 250 mg tab were from Zydus Cadila. Tirofiban (AGGEBLOC) 5mg/100ml was from Nicholas Piramal.
Chemicals and reagents

The chemicals and reagents were procured from RANKEM India (Pvt) Ltd, Mumbai, TULIP Diagnostic India (Pvt) Ltd, Mumbai and HiMEDIA Diagnostic India (Pvt) Ltd, Mumbai. Sodium citrate solution, diluting fluid, saline solution, surgical spirit was from RANKEM, fibrinogen reference was from HiMEDIA and calcium chloride was from TULIP.

Grouping of animals

Swiss albino mice of either sex, weighing 20-25 Gms were used in present study and were acclimatized under standard housing conditions. The animals had free access to water and rat food. The animals used for study were approved by the Institutional Animal Ethical Committee (IAEC) of SRM college of Pharmacy, SRM UNIVERSITY, Kattankulathur, Kancheepuram (Dist).

In the present study 24 animals were used and are grouped as four with six in each (group-A, B, C&D).

- **Group-A**: treated with Aspirin (10 mg/kg, orally).
- **Group-B**: treated with Aspirin and Clopidogrel (10 mg/kg, orally).
- **Group-C**: treated with Ticlopidine and Tirofiban (1mg/kg, 0.4mcg/kg/min, orally and I.V.).
- **Group-D**: treated with Tirofiban and Dipyridamole (0.4mcg/kg/min, 1mg/kg, I.V. and orally).

Experimental work

The animals were fasted for overnight, at the time of 8 am to 9 am each group of animals were treated with respective drugs through specified routes. Then the animals were observed for 2 hours after 2 hours the venous blood was collected from the tail vein of individual animals and were analyzed for following parameters: Bleeding time [7,8], Clotting time [9], Total platelet count (10^3 cells/cu, mm), Thrombin content (mcg/ml), Rate of thrombin generation (mcg/ml/min), weight of fibrin (mcg/ml) / time taken for fibrin formation (min) and Thrombin generation time (min) [10, 11,12].

RESULTS AND DISCUSSION

Tirofiban+ ticlopidine combination of drugs prolonged the duration of bleeding time when compared to treatment with aspirin alone and other drug combinations. But Tirofiban + Dipyridamole combination extends the clotting time to a greater extent than that of Tirofiban + Ticlopidine which is better when compared to other combinational drugs.

The graphs 1.3,1.4 &1.6 clearly indicates that the Tirofiban+ Ticlopidine is effective in delaying thrombin generation and there is a decrease in total platelet count after treatment and we can say that it can also delay platelet aggregation. But there is no notable changes in fibrin content after treatment with these combinational drugs.

After comparative study of antiplatelet drug combinations with each other, the bleeding time and clotting time data gives the effect on the platelet activity may be due to the decrease in the platelet count or platelet cell efficiency in the primary hemostasis mechanism causing the increase in the bleeding & clotting time. Tirofiban+ Ticlopidine combinational drug treatment shows the considerable change in the bleeding time & clotting time. Tirofiban+ Dipyridamole & Aspirin+ Clopidogrel combinational drug treatments show the lesser change than the Tirofiban+ Ticlopidine.

Treatment with aspirin shows considerable decrease in the Total platelet count than Aspirin+ Clopidogrel & Tirofiban+ Dipyridamole combinational drug treatment. Tirofiban+ Ticlopidine treatment gives more effect on Total platelet count than the Aspirin treatment.

![FIG. 1.1: Effect of Aspirin, Aspirin and Clopidogrel, Ticlopidine and Tirofiban & Tirofiban and Dipyridamole on bleeding time.](image)
Fig. 1.2: Effect of Aspirin, Aspirin and Clopidogrel, Ticlopidine and Tirofiban & Tirofiban and Dipyridamole on clotting time

Fig. 1.3: Effect of Aspirin, Aspirin and Clopidogrel, Ticlopidine and Tirofiban & Tirofiban and Dipyridamole on total platelet count (no. of cells/cu.mm)

Fig. 1.4: Effect of Aspirin, Aspirin and Clopidogrel, Ticlopidine and Tirofiban & Tirofiban and Dipyridamole on thrombin generation time (mins)

Fig. 1.5: Effect of Aspirin, Aspirin and Clopidogrel, Ticlopidine and Tirofiban & Tirofiban and Dipyridamole on formed fibrin content.
The Tirofiban+Ticlopidine drug combinational treatment delays the thrombin generation but no change was observed in the formed thrombin content volume, there is decrease in thrombin generation than the Aspirin & Aspirin+Clopidogrel drug treatment. Tirofiban+Dipyridamole also show considerable decrease in the thrombin generation. Increased thrombin generation rate is useful in the Acute Coronary Syndrome (ACS) and Acute Myocardial Infarction (AMI) treatment and management.

CONCLUSION

In Acute Coronary Syndrome (ACS) and Acute Myocardial Infarction (AMI) treatment and management various antiplatelet drugs are used for delaying the primary hemostasis events using antiplatelet drugs causing stimulation of secondary hemostasis by initiating thrombin generation. This is the undesired event in the clinical ACS & AMI treatment. The present study was concluded as combinational antiplatelet drugs considerably delays the thrombin generation than the single drug treatment. Tirofiban+Ticlopidine & Tirofiban+Dipyridamole significantly delay the thrombin generation than the single drug Aspirin and Aspirin+Clopidogrel combinational drug treatment.

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