SYNERGISTIC INTERACTION OF ADENOSINE DIPHOSPHATE – EPINEPHRINE AND EPINEPHRINE – COLLAGEN IN AGGREGATION OF HUMAN PLATELETS

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Background: Efficient hemostasis in human body depends on a complicated series of events which actively involve blood platelets. Platelets exhibit diverse responses in a variety of agonists. In vivo, most of the agonists act in synergism, causing aggregation of platelets. **Methods:** In this study, the synergism between ADP – Epinephrine and Epinephrine – Collagen has been determined by means of turbidometric method, which measures changes in optical density of platelet suspension. The study was carried out at the department of Hematology, Armed Forces Institute of Pathology, Rawalpindi. The subthreshold concentrations of each of the agonists were established with the help of dose response curve. By adding these agonists in subthreshold concentrations, the synergism between ADP – Epinephrine and Epinephrine – Collagen was determined. **Results:** The combinations of these agonists in subthreshold levels showed the synergistic responses in causing platelet aggregation. **Conclusion:** These findings indicate that the optimal platelet aggregation does occur between the above mentioned pairs of agonists even when each of the agonist is added to the other in subthreshold doses.

Keywords: Platelet aggregation, agonist, synergism, Adenosine diphosphate, Collagen, Epinephrine.

INTRODUCTION

Platelets are small granulated, anucleate cell fragments circulate as cytoplasmic discs of 6-8fl[1]. Platelets have a critical role in the response to injury that involves the process of hemostasis, thrombus formation, vascular and connective tissue healing[2]. Recent studies have revealed a definite role of platelets in the pathogenesis of atherosclerosis[2].

Platelets in contact with damaged or disrupted endothelium become activated, change their shape from normal discoid shape to spiny sphere with long thin filopodia, extending several micrometers out from the platelet and ending in points[3]. They also release the contents of their granules during activation. These factors stimulate the proliferation of smooth muscle cells in the intima of arteries, promote the migration of fibroblasts from media to the intima of the vessel wall, and aggregate the activated platelets to one another[4].

There have been studies reporting increased platelet aggregation and increased response to agonists stimulation in vitro[5,6,7]. Platelet activation in vivo probably involves a combination of agonists, with perhaps collagen more important at the beginning, thrombin more important later on, and with the other agonists in varying mixture throughout[8,9].

The present study was designed to evaluate the possible synergistic interaction of subthreshold concentrations of ADP – Epinephrine and Epinephrine – Collagen in aggregation of human platelets.

SUBJECTS AND METHODS

This experimental study was conducted on 120 samples of platelets at department of Hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. These samples were isolated from healthy, non-smokers, non-hypertensive, non-diabetic volunteers who were in age group of 20-50 years and not on medications for at least last 15 days that is known to interfere with platelet function.

Subjects were evaluated by taking detailed history, performing general and physical examination and doing laboratory investigations like bleeding time, platelet count, blood glucose, serum urea, serum creatinine and urine for glucose and proteins. All the subjects gave informed consent before the study.

Platelet Aggregation Studies: Fasting venous blood was collected with minimal venous occlusion in a plastic conical centrifuge tube containing 3.8% sodium citrate in a ratio of blood to anticoagulant of 9:1. Precautions were taken to avoid stasis and contamination with tissue fluids.

The anticoagulated blood in centrifuge tube was centrifuged at 1500 revolutions per minute (rpm) for 15 minutes at room temperature. The resultant platelet rich plasma (PRP) was carefully transferred to a test tube labeled "PRP". The remaining anticoagulated blood was recentrifuged at 4000 rpm for 5 minutes to separate platelet poor plasma (PPP). A platelet count was preformed on PRP and it was adjusted to 350,000 per microliter $\pm$ 50,000 with PRP as needed.

Platelet aggregometer and a chart recorder were switched on to warm up the heater block upto 37°C and stirring speed was fixed to 1100 rpm. 500 µL of PPP in a glass cuvette was placed in a well marked 'PPP' and 450 µL of PRP in another glass cuvette was placed in a well labeled 'PRP' after adding a magnetic stirring bar. Platelet aggregation was measured by using a platelet aggregometer (Chronolog Corporation, USA) which works on turbidometric method described by Born[10], and change in light transmittance was recorded on Omniscribe Chart Recorder.

The reagents used in the study to aggregate platelets were Adenosine diphosphate in concentrations of 1.0, 1.5, 2.0 and 3.0 μmol/L, Epinephrine in concentrations of 0.3, 0.4, 0.5 and 1.0 μmol/L, and Collagen in concentrations of 2, 3, 5 and 10 μg/ml. after taking the baseline by using its button, the aggregation response was recorded by adding 50 μL of each of the aggregating reagents to each cuvette containing PRP. The aggregation response was interpreted as intensity of aggregation using the technique explained by Roper et al[11].

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RESULTS

The result of the study showed the mean subthreshold values of;

- Collagen as 3 μg/ml, as shown in fig-1.

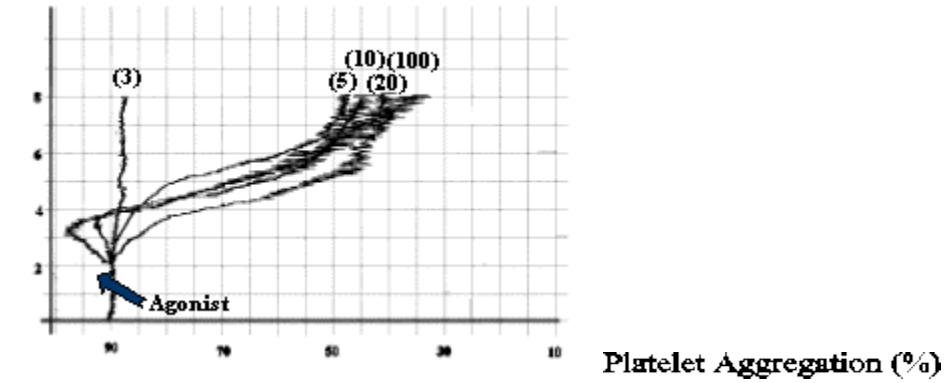
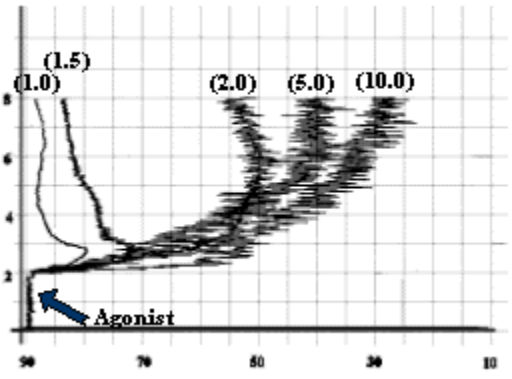


Fig-1:Platelet aggregation by different concentrations (μg/ml) of collagen.

Time (minutes)

- ADP as 1.5 μmol/L, as shown in fig-2.



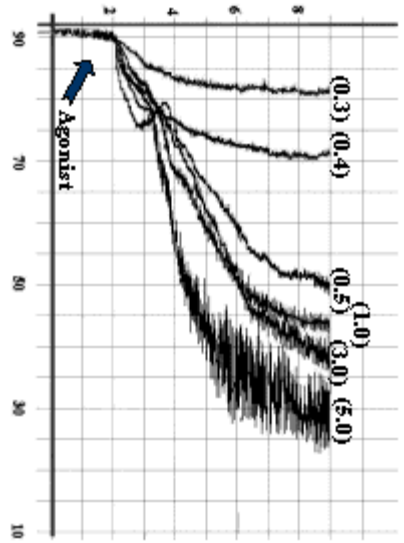
Time (minutes)

Platelet Aggregation (%)

Fig-2: Platelet aggregation by differentconcentrations (μmol/L) of ADP.

- Epinephrine as 0.4 μmol/L, as shown in fig-3.

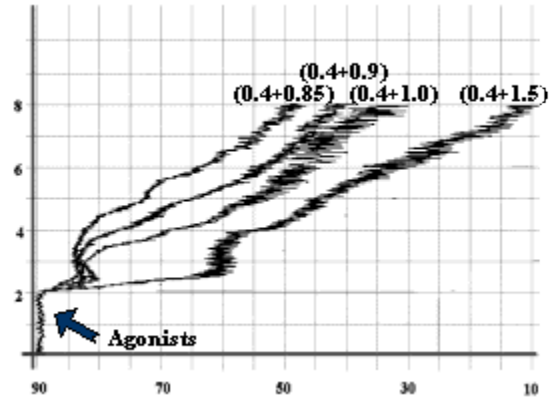
Time (minutes)



Platelet Aggregation (%)

Fig-3:Platelet aggregation by different concentrations (μmol/L) of Epinephrine.

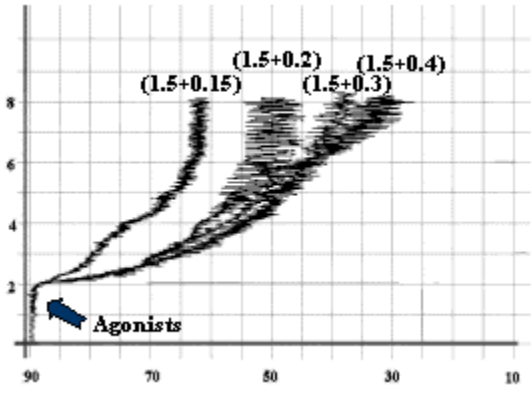
And the combinations of ADP – Epinephrine and Epinephrine – Collagen in their subthreshold concentrations showed the synergistic responses in causing platelet aggregation, as shown in figure-4, 5, 6 and figure 7.



Platelet Aggregation (%)

Time (minutes)

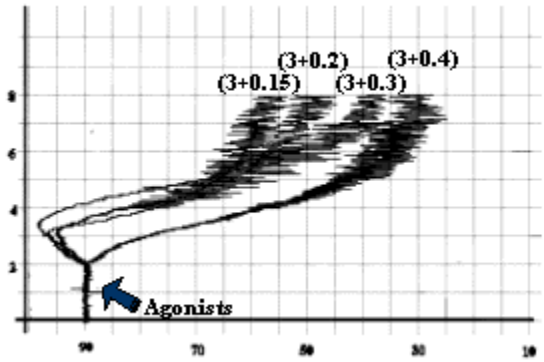
Fig-4: Platelet aggregation by subthresholdconcentration (0.4μmol/L) of Epinephrineand subthreshold concentrations (1.5, 1.0, 0.9 and 0.85μmol/L) of ADP.



Time (minutes)

Platelet Aggregation (%)

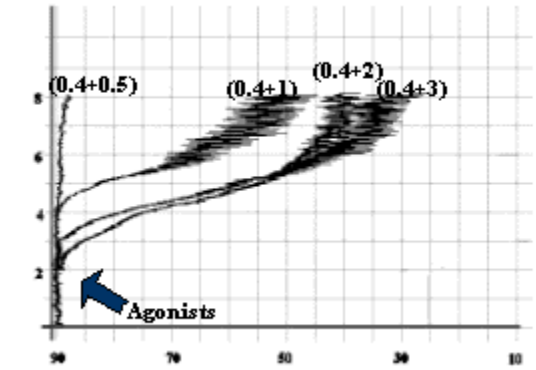
Fig-5: Platelet aggregation by subthresholdconcentration (1.5μmol/L) of ADP andsubthreshold concentrations (0.4, 0.3, 0.2 and 0.15μmol/L) of Epinephrine.



Time (minutes)

Platelet Aggregation (%)

Fig-6: Platelet aggregation by subthresholdconcentration (3μg/ml) of collagen andsubthreshold concentrations (0.4, 0.3, 0.2 and 0.15μmol/L) of epinephrine.



Time (minutes)

Platelet Aggregation (%)

Fig-7: Platelet aggregation by subthresholdconcentration (0.4μmol/L) of epinephrineand subthreshold concentrations (3, 2, 1 and 0.5μg/ml) of collagen.

DISCUSSION

In the study, only pairs of more common agonists were studied, since extension to combinations of more agonists would involve a nearly endless task. We have shown that the aggregation response induced by epinephrine in combination with ADP was synergistic. Other studies have demonstrated synergism between these platelets agonists[12,13,14]. Ardlie et al[15] first reported that epinephrine not only caused platelet aggregation but also enhanced the aggregation induced by other agonists, like ADP. Venag et al mentioned the marked enhancement of ADP induced aggregation on addition of subthreshold concentration of epinephrine[16].

The result of present study also showed the synergistic response in aggregating platelets by epinephrine and collagen when added together simultaneously in low concentration. The result of this study is also in agreement with the result of Huang and Detwiler, who demonstrated the potentiated response to platelet aggregation on combination of collagen and epinephrine. They also stated that the pattern of responses was intermediate between that typical for either agonist alone when neither agonist was in relatively higher concentration[17].

Thus, the results of the study revealed that the optimal platelet aggregation does occur between the above mentioned pairs of agonists even when each of the agonist is added to the other in subthreshold doses. Each of these agonists has its own specific receptor on platelet surface with specific intracellular signal transduction mechanism[18,19,20]. As their receptors and associated intracellular signaling pathways in synergistic responses of these agonists have not been investigated in the study, this aspect remained unclear.

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CONCLUSION

We may conclude in agreement with other investigators that the synergistic potentiation of some of the agonists in subthreshold concentrations in vivo may be responsible for the activated state of platelets and their complications as observed in essential hypertension, diabetes mellitus, ischemic heart disease, and transient ischemic attacks. However, the biochemical basis and intracellular signaling of these synergistic responses needs further exploration.

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