INTERACTIONS BETWEEN RESVERATROL AND TIROFIBAN ON PLATELET FUNCTION, BLOOD PRESSURE AND LIPID PEROXIDATION, AN EXPERIMENTAL STUDY.

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Summary

The intravascular coagulation is one of the serious conditions. The antiplatelet agent like tirofiban is indicated in such few conditions like unstable angina, after angioplasty to prevent thrombus formation. Bleeding is one of the most common conditions requiring clinical monitoring during the therapy. Resveratrol, a bioactive agent in vegetarian diet, especially in red wine, has been reported to possess antiplatelet activity. Thus it may be expected to interact with tirofiban if consumed resveratrol rich diet during the therapy. The interaction between tirofiban and resveratrol was studied for their effect on platelet aggregation, blood pressure as well as antioxidant activity. The results showed that the co administration of tirofiban with resveratrol potentiated mutually the anti platelet activity and the combination was effective antihypertensive and possessed significant ($p<0.01$) antioxidant activity.

Keywords: Antiplatelet; Blood pressure; Lipid peroxidation; Resveratrol; Tirofiban.

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Introduction

Intravascular coagulation is one of the serious conditions and depending upon the site of involvement it may be life threatening *viz.* unstable angina, myocardial infarction, *etc.* Drug therapy in such situation aims to arrest or lyse the thrombus and to maintain meticulous balance between endogenous coagulant and anticoagulant mechanisms. Platelet activation and aggregation, an initial steep in thrombus formation can be inhibited by antiplatelet agents acting by various mechanisms. These antiplatelet agents like aspirin, clopidogrel, ticlopidine are used for long term prophylaxis against thrombosis, while tirofiban, abciximab, eptifibatide along with heparin for short term prophylaxis as in unstable angina, after angioplasty or atherectomy, *etc.*[1]

Combination therapy of antiplatelets for prophylaxis is obviously to ensure therapeutic efficacy and needs monitoring to avoid bleeding complications. Resistance development for antiplatelet action of aspirin, genetic polymorphism associated with clopidogrel resistance[2] and their slow onset of action are their major limitations. Resveratrol, a major bioactive agent[3] has been reported for its anti-inflammatory, anti-oxidant, vasodilator and anti-platelet acitivities[4]. In contrast to tirofiban it is orally effective and could be expected to augment antiplatelet activity of tirofiban, if co administered. Similarly it could be hypothesized that an individual on antiplatelet therapy might develop bleeding complications by consuming resveratrol rich fruits. Due to paucity of information about such interactions the present study was planned to investigate interaction between resveratrol and tirofiban using human platelet rich plasma (PRP) from healthy blood donors.

Since resveratrol has been reported to possess vasodilatory effect it is likely to influence the BP, which is an important parameter often deciding the therapeutic outcome in cardiovascular diseases. Therefore, in the present study resveratrol and tirofiban were investigated for their effect on conscious BP of male Sprague-Dawley rats.

Materials and methods

Drugs and chemicals:

Resveratrol and Nitric oxide colorimetric estimation kit (cayman chemicals, USA), Tirofiban (Zydus research centre, Ahmedabad), Cholic acid and Propyl thiouracil (Sigma-Aldrich, USA).

In-vitro platelet aggregation test:

About 10ml of blood samples with 3.8% of sodium citrate were taken while donating the blood in blood bank from healthy blood donors who consented for platelet function test and the study was approved by IRB. The samples were divided into different groups, and subjected to platelet aggregation test using aggregometer as described earlier[5]. The preliminary studies showed adenosin diphosphate (ADP) of 10µg induced 100% aggregation of 0.45ml of sample and the same dose was used in the study. (Fig 1a) The effect of pretreatment for 5min with different drugs on ADP induced platelet aggregation were studied by subjecting 0.45ml PRPs obtained from each treated sample to aggregometer. The effect was expressed as percentage inhibition of ADP induced platelet aggregation.

In-vivo evaluation:

Animals

Healthy male Sprague-Dawley rats weighing 200±20g were acclimatized to laboratory with free access to standard pellets (Amrut brand) and water for a week. The rats were divided into five groups. After a week, four of the groups were fed with 1ml of high fat diet (HFD) containing cholesterol, cholic acid, propylthiouracil and soyabean oil to induce hypertension.[6]

The study was approved by Institutional Animal Ethics Committee constituted as per the CPCSEA guidelines, New Delhi.

The different treatment received by each group were

Group 1. standard diet (negative control)

Group 2. High fat diet (positive control)

Group 3. HFD + Resveratrol

Group 4. HFD + Tirofiban

Group 5. HFD + Resveratrol + Tirofiban

The treatment was continued for 12 days. On day 13 SBP was measured and blood sample was collected through cardiac puncture under light ether anaesthesia for biochemical analysis. The rats were sacrificed humanely.

Systolic Blood Pressure[7]: Rats were acclimatized for about 5-6 hr in lab at room temperature (30 ^{0}C). SBP was measured with a tail-cuff sphygmomanometer (Harvard apparatus, USA) between 15:30 and 16:30 h.[8] The equipment used included a restrainer, a tail cuff containing latex tube and a dual – channel recorder. Rats were allowed to acclimatize in restrainer before measuring SBP and then tail –cuff was placed on the rat tail and moved towards base till the sensors detects pulses. Then pressure was applied and systolic blood pressure was determined at least 8 times in each animal using Biopac system inc. (MP100A-CE 111A4306, Santa Barbara, California) to calculate group mean. The mean of eight consecutive readings was used for statistical comparisons.

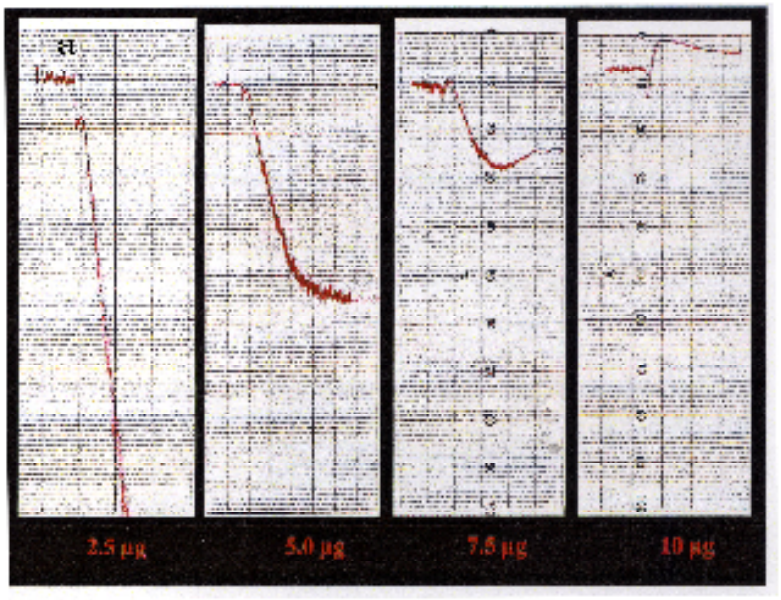
Estimation of lipid peroxide: Thiobarbituric acid reactive substance (TBARS) method was used to measure malondialdehyde (MDA) as described earlier[9].

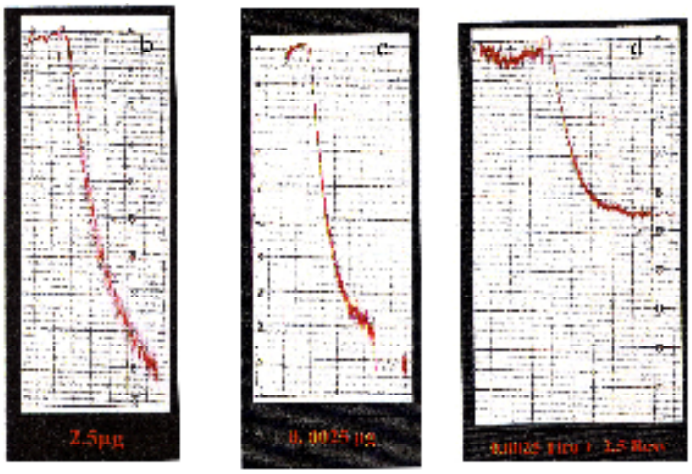
Estimation of nitric oxide (NO): Nitric oxide colorimetric estimation kit was used for estimation as described earlier[10].

Statistical analysis

The results were analysed by ANOVA followed by post hoc Dunnet's posthoc test and $p \leq 0.05$ was considered as significant.

Fig 1: Strip chart tracing of ADP induced platelet aggregation & its inhibition by different treatments





a. Different concentrations of ADP , b. Resveratrol c. Tirofiban
d. Resveratrol + Tirofiban

Results

In-vitro platelet aggregation test

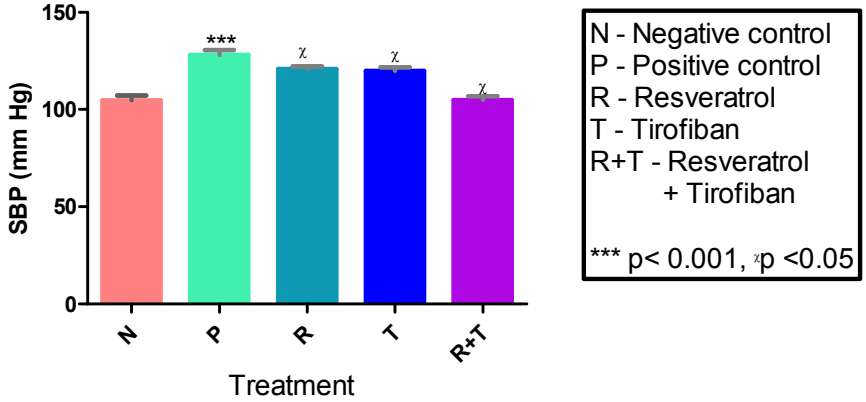
Resveratrol at the concentration of 2.5µg the inhibition of ADH induced platelet aggregation was 5% (Fig 1b), whereas at 15µg it was 100% and pre incubated with 0.0025µg concentration of tirofiban 5% inhibition of platelet aggregation was seen (Fig 1c), while at 0.01µg concentration it completely (100%) inhibited platelet aggregation.

In order to study the interaction on platelet aggregation least concentration of resveratrol (2.5µg) and tirofiban (0.0025µg) which individually produced negligible inhibition (5% each) were combined. The pre incubation with the combination produced 53% inhibition, indicating synergistic interaction between the two. (Fig 1d)

In vivo evaluation

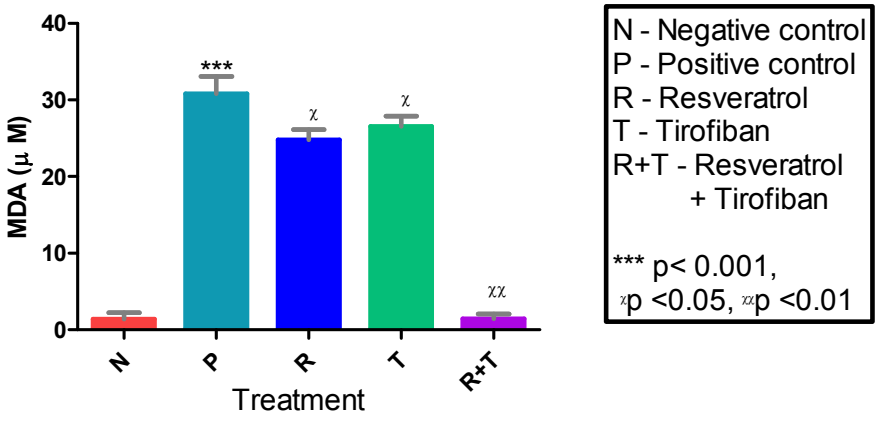
Systolic Blood pressure (SBP) equivalent to mm of Hg: The positive control (high fat diet fed) group with mean SBP, 128.2±2.33 was significantly ($p<0.001$) increased compared to that of negative (normal diet fed) control (104.8±2.30). The groups pretreated with resveratrol, tirofiban and their combination the mean SBP was 120.8±1.44, 119.9±1.69 and 105.0±2.02 respectively were comparable to that of negative control but significantly ($p<0.05$) decreased compared to that of positive control.(Fig.2)

Fig 2. Effect of various treatment on Systolic Blood Pressure



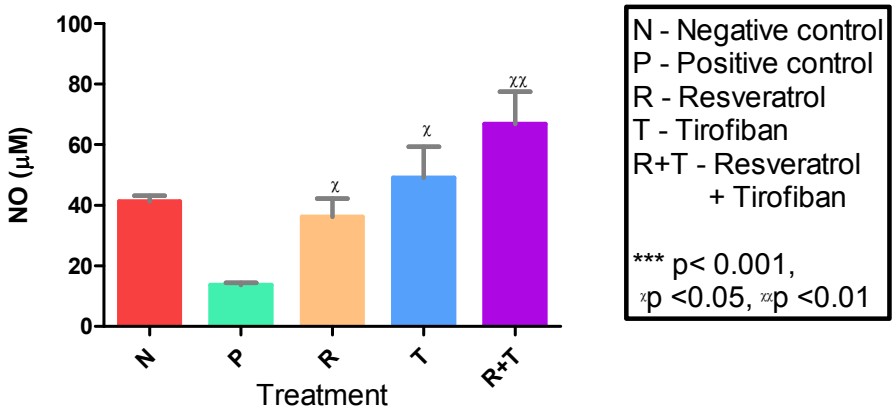
Lipid peroxidation assay: MDA estimated as a marker of lipid peroxidation showed a significant ($p<0.001$) increase with a mean of 30.8±2.22µM compared to that of negative control with mean 1.4±0.81µM. the groups pretreated with resveratrol and tirofiban with mean MDA of 24.8±1.32 and 26.6±1.34 were significantly ($p<0.01$) reduced compared to that of positive control, while in groups pretreated with combination (resveratrol + tirofiban) the mean MDA was 1.5±0.59 almost comparable to that of negative control and significantly ($p<0.001$) reduced as compared to that of positive control group. (Fig 3)

Fig 3. Effect of various treatment on MDA



Nitric oxide estimation: the mean value in µM of 36.2±6.06 and 49.1±10.3 in groups treated with resveratrol and tirofiban respectively were significantly ($p< 0.05$) increased as compared to 13.7±0.75 of positive control. The mean value of 66.8±10.75 of resveratrol and tirofiban combination treated group was not only significantly ($p<0.001$) higher than that of positive control but also of negative control indicating synergistic activity of these drugs. (Fig 4).

Fig 4. Effect of various treatment on Nitric Oxide



Discussion

The findings of the present study clearly indicate that resveratrol in a concentration dependent manner inhibits platelet aggregation and this finding agrees with the earlier report.[11] Tirofiban similar to resveratrol suppressed platelet aggregation in a dose dependent manner and its anti platelet activity has been attributed to its antagonistic activity at glycoprotein (gp) IIb/IIIa receptor.[12] Resveratrol and tirofiban in concentration (2.5 and 0.0025µg) to produce minimal platelet aggregation (5%), when combined together produced significant anti-platelet activity (53%) indicating their synergistic interaction. Literature survey indicates that, there is paucity of information regarding such an interaction between resveratrol and tirofiban.

Resveratrol produced anti hypertensive effect in animals rendered hypertensive with high fat diet and such anti hypertensive activity of resveratrol in fructose fed rats has been reported.[13] Similarly tirofiban in the dose of 5µg/kg significantly reduced blood pressure in animals. The anti hypertensive activity of tirofiban as observed in the present study has not been documented. Combination of equimolar doses of resveratrol and tirofiban lowered the blood pressure to basal level, indicating their synergistic anti hypertensive activity. Such an interaction between resveratrol and tirofiban has not been reported.

Both resveratrol and tirofiban individually and in combination increased bioavailability of nitric oxide in animals after 12 days of treatment. Resveratrol and tirofiban have been reported to increase eNOS activity leading to increased availability of NO.[13,14]

Both resveratrol and tirofiban individually and together significantly reduced MDA levels indicating their inhibitory effect on lipid peroxidation. Resveratrol has been reported to suppress TBARS in platelets,[11,15] while there is scanty information on such an activity of tirofiban.

Anti-platelet activity of tirofiban is said to be due to blockade of gpIIb/IIIa receptor, while exact mechanism of how resveratrol prevents aggregation is not known. However, gpIIb/IIIa as a site of action for resveratrol seems to be unlikely, as the concentration of resveratrol required to produce similar degree of anti-platelet action as that of tirofiban was about 1000 times. Moreover mutual potentiating and not additive effect observed in the present study indirectly suggest that resveratrol targets on the platelets are not exclusively gpIIb/IIIa receptors. Though the study was not planned to probe into the nature of interaction between resveratrol and tirofiban it appears to be of pharmacodynamic in nature as the present findings on platelet aggregation were based on the *in vitro* studies.

Similar interaction between resveratrol and tirofiban (as observed in the present study) are likely to be encountered in clinical practice, since patients on tirofiban therapy may consume beverages like grape juice, red wine, etc., which are reported to be rich sources of resveratrol. Since tirofiban is used for a short period in unstable angina (acute

coronary syndrome) such an interaction may not be of clinical significance, moreover pharmacokinetic data regarding resveratrol; particularly the duration of antiplatelet activity is not well documented. Resveratrol from the diet may not be sufficient to elicit pharmacological effects, but consumption of beverages (red wine) containing significant amount have been claimed to lower cardiac mortality[4], as described in French people (French paradox).[16] The anti-oxidant activity of resveratrol as confirmed in the present study also be partly contributing for such a beneficial effect.

Tirofiban has limited value in the treatment of acute myocardial infarction and has been administered along with heparin. The findings of the present study indicates that resveratrol could be a substitute for heparin in such conditions and the same needs to be evaluated clinically.

In clinical practice anti-platelet therapy is carefully titrated so as to avoid intravascular clotting without causing bleeding complications. Addition of any agent that interferes with the platelet activity is likely to upset this balance leading to complication either in the form of bleeding or intravascular clotting.

The observations of the present study hint the possible interactions of resveratrol rich diet or beverages (non medicinal resveratrol) with various antiplatelet and antihypertensive agents and suggest clinical monitoring for such outcomes in order to ensure patient safety.

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References

1. Brunton LL, Laso JS, Parker KL, Goodman Gilman A. The Pharmacological Basis of Therapeutics. 11th ed. New York : McGraw Hill; 2001.
2. Anthony S. Fauci, Eugene Braunwald, Dennis L, *et al.* Harrison's Principles of Internal Medicine. 17th ed. Vol 2. New York: McGraw Hill; 2008.
3. Weil AVD, Golde PHMV, Hart HC. Blessings of grape. Eur J Internal Med 2001; 12: 484-9.
4. Fremont J. Biological effects of Resveratrol. Life Sci 2000; 66(8): 663-73.
5. Paul H. platelet function analysis. Blood Reviews 2005; 19(2): 111-23.

6. Badyal DK, Lata H, Dadhich AP. Animal models of hypertension and effects of drugs. Ind J Pharmacol 2003; 35: 349-62.

7. Bunag RD, Butterfield J. Tail-cuff blood pressure measurement without external preheating in aware rats. Hypertension 1982; 4: 898-903.

8. Patil BM, Kulkarni NM, Unger BS. Elevation of systolic blood pressure in an animal model of olanzapine induced weight gain. European Journal of Pharmacology 2006; 551: 112-115.

9. Placer, Z.A., L.L. Cushman and B.C. Johnson, 1966. Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. Anal. Biochem 16: 359-364.

10. Nims RW, Darbyshire JF, Saavedra JE Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions. Methods 1995, *7:* 48-54.

11. Olas B, Wachowichz B, Juszczak JS, Zeilinski T. Effect of Resveratrol, a natural polyphenolic compound, on platelet activation induced by endotoxin or thrombin. Thromb Res 2002; 107: 141-5.

12. Llveskero S, Lassila A. Differential inhibitory effects of platelet glycoproteins IIb/IIIa antagonists on aggregation induced by procoagulant agonists. Thromb Res 2006;117(3):291-7.

13. Miatello R, Vazquez M, Renna N, Cruzado M, Zumino AP, Risler N. Chronic administration of Resveratrol prevents biochemical cardiovascular changes in fructose fed rats. Am J Hypertens 2005; 18: 864-70.

14. Yang YJ, Zhao JL, You SJ, Wu YJ, Jing ZC, Xang WX, Meng L, Wang YW, Gao RL. Different effects of tirofiban and aspirin plus clopidogrel on myocardial no-reflow in a mini-swine model of acute myocardial infarction and reperfusion. *Heart* 2006;92 :1131-1137.

15. Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural anti oxidant derived from grapes. Cardio Res 2000; 47: 549-55.

16. Law M, Wald N. Why heart disease mortality is low in France: the time lag explanation. Br Med Journal May 1999; 318(7196): 1471–1480.