Original Article

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Pharmacology Section

A Pilot Study on the Effect of Angiotensin Receptor Blockers on Platelet Aggregation in Hypertensive Patients- A Prospective Observational Study

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ABSTRACT

Introduction: Thrombosis is an invariable component contributing to cardiovascular events in patients with hypertension. One of the risk factors of cardiovascular disease is increased platelet activity. One among the widely used antihypertensive agents are Angiotensin II type 1 Receptor Blockers (ARBs). Even though there are many studies involving antihypertensive agents, their antithrombotic properties remain elusive and not fully characterized.

Aim: To evaluate the anti-aggregatory effect of ARBs on platelets in-vivo.

Materials and Methods: A total of 60 subjects were included in this observational pilot study conducted in the medicine out patient department of JJM Hospital, Davanagere, Karnataka, India. Among them, 30 patients with essential hypertension attending Medicine OPD of a tertiary care hospital, who were on ARB for at least one month, were enrolled into study group. The control group consisted of 30 normotensive subjects who were not on any drug affecting platelet function. The Bleeding Time (BT) was evaluated for both the groups using Duke method of BT estimation. Data was analysed using SPSS software version 20. The test group was compared with control group using student's unpaired t-test.

Results: The mean BT of study group was 2.488 minutes ± 0.0361 Standard Error of Mean (SEM) and that of control group was 1.998 minutes ± 0.0362 SEM. The result was statistically significant ($p<0.001$). The average duration of treatment was 2.933 years.

Conclusion: ARB have antiplatelet activity. Increase in BT in ARB group when compared with that of control group is a reflection of antiplatelet activity.

Keywords: Antiplatelet activity, Bleeding time, Thromboxane A2

INTRODUCTION

In hypertension there is an increased pressure on arterial vasculature [1]. This change in shear stress is one of the causes of endothelial injury. Endothelial injury makes the vascular lumen susceptible to thrombus formation. Exposed sub-endothelial collagen is a platelet activator and promotes platelet adhesion to the injured site [2]. The formation and development of atherosclerosis in medium and large vessels is promoted by hypertension [1,3]. Thrombus is the pathologic extension of haemostasis. Platelets play a pivotal role in primary haemostasis and involve transformation of platelet into haemostatic plug through adhesion, platelet granule release reaction, platelet aggregation and consolidation [4]. In thrombosis coagulation reactions are unregulated and results in abnormal enlargement and occlusion of lumen wall. This is the pathological basis of hypertension causing cardiovascular and cerebrovascular events resulting in end organ damage in heart, brain and kidneys [1,5].

Platelets release chemotactic factors/cell chemokines that induce monocytes, macrophages and endothelial cells to release Tumour Necrosis Factor (TNF) and cell chemokines that in turn act on respective receptors on platelets thus forming a vicious cycle which is one of the mechanism for the formation of unstable plaque [6,7]. Thus it is clear that platelets have a definitive role right from the formation of atherosclerosis to the cardiovascular and cerebrovascular events. Hence antiplatelet activity is beneficial in hypertensives and inhibition of platelet aggregation has become a critical step in preventing thrombotic events [8].

Angiotensin II type 1 Blockers (ARBs) are commonly used for the treatment of hypertension [9]. They have tolerability and safety profile better than that of Angiotensin Converting Enzyme (ACE) inhibitors [10]. ARBs have antiplatelet activity [9]. ARBs like Losartan, Irbesartan, Telmisartan and Valsartan are known to exhibit antiplatelet activity in-vitro [11]. Antiplatelet aggregation of these agents could be of additional benefit in hypertensive patients and is desirable in hypertensive patients with high atherothrombotic and/or thromboembolic risk [9]. Bleeding time (BT) is a laboratory test that can be used to assess platelet function [12,13]. BT is inexpensive and does not need expensive equipment. It is unaffected by the method of sampling and anticoagulants. The results are almost readily available and only a small amount of blood is needed [14]. The present study aims to demonstrate the antiplatelet aggregatory activity of these agents in-vivo using Duke method of BT estimation.

MATERIALS AND METHODS

It was an observational pilot study conducted in the medicine outpatient department of JJM Hospital, Davanagere, Karnataka, India. The study duration was for a period of six months from July 2015 to January 2016. Ethical clearance was taken from the institutional ethical committee before conducting the study. Written informed consent was taken from all subjects. Since this was a pilot study and there were no other similar study in the past which could provide data to calculate sample size, we had estimated the sample size using the rule of thumb method for pilot studies [15]. Hence, 30 patients with essential hypertension attending medicine OPD for follow-up and on ARB for atleast one month were enrolled into study group. Thirty normotensive healthy volunteers who were accompanying patients and who were not on any drug affecting platelet function were enrolled into control group.

Patients with secondary hypertension, co-morbid bleeding disorders, thrombocytopenia, fever, uraemia, coagulation disorders, who were on medications that could potentially affect platelet activity (NSAIDs, hypolipidemics, antiplatelet drugs, heparin, fibrates) and pregnant women were excluded from the study.

The BT was evaluated for both the groups using Duke method of bleeding time estimation. Duke method is similar to the Ivy method, but here blood pressure cuff is not required. The advantage of Duke method is that it is less invasive, since it involves making a puncture wound that is 3mm deep after sterilizing the area with alcohol [12,13]. Left middle fingertip was pricked with a lancet. The wound was swabbed with a filter paper every 15 seconds until the blood was no more absorbed. Standard filter paper was used. A single lab technician, who was trained to perform the test, conducted all the BT estimation. All the tests were done twice and their mean was considered as observed value.

Results were expressed in terms of means ± SEM. Data being continuous variable, statistical test was done by student's unpaired t-test to compare test group with control group. The p-value of <0.05 was considered statistically significant. Data was analysed using SPSS software version 20.

RESULTS

The base line characteristics of both the groups are given in [Table/Fig-1]. As shown in [Table/Fig-2], the mean BT of study group was statistically significant compared to control group. The p-value <0.001. Average duration of treatment of ARBs was found out to be 2.933 years.

Parameters	Study Group (ARBs)	Control Group
Age (in years)	53.467	51.933
Sex		
Males	16	15
Females	14	15
SBP	130.333	121.667
DBP	87.333	83.333
Average Dose (mg/day)		
Losartan	30	-
Telmisartan	37.142	
Olmesartan	40	
Average duration of treatment (in years)	2.933	-

[Table/Fig-1]: Baseline characteristics of subjects.
SBP - Systolic Blood Pressure
DBP - Diastolic Blood Pressure

Groups	Mean BT**	SD	SEM
Study Group	2.4880	0.1978	0.0361
Control Group	1.998	0.1984	0.0362

[Table/Fig-2]: Mean bleeding time comparison.
** Mean Bleeding Time expressed in minutes.
p-value <0.001

DISCUSSION

The results of this study are in accordance with previous studies on antiplatelet effect of ARBs, including a study employing laser light scattering method [16]. In another study ARB losartan suppressed ex-vivo platelet activation in washed human and canine platelet suspensions [17]. Among the ARBs in this study Losartan accounted for 67%, Telmisartan 23% and Olmesartan 10% of the study group. Losartan is one of the most commonly prescribed ARB and also the first drug approved in this category. It has also given insight into the physiology of angiotensin II and the clinical importance of its blockade [18]. Inhibition of human platelet thromboxane A2/Prostaglandin H2 is a possible mechanism of action of Losartan. The metabolite of Losartan, EXP 3174 is also known to react with Thromboxane A2 [16,19]. Thromboxane A2 (TXA2) is a potent inducer of platelet aggregation and platelet granule release reaction. Increased intracellular concentration of cAMP in platelets leads to decreased platelet aggregability. Physiologically platelet cAMP levels are regulated by TXA2 and PGI2. Although the exact mechanism by which cAMP leads to decreased platelet aggregation is unknown, it is known that increase in cAMP causes activation of protein kinase A which through incompletely elucidated mechanism causes a decrease in intracellular calcium in platelets. Increase in intracellular calcium is essential for platelet aggregability [20]. Antiplatelet aggregation of these agents could be of additional benefit in hypertensive patients and is desirable to treat hypertensive patients with high atherothrombotic and/or thromboembolic risk [9]. Duke method of BT estimation was employed in this study. Even though BT is a basic investigational modality it is an indicator of platelet function [12,13]. The objective of our study was to ascertain whether ARBs have antiplatelet activity. Since our study was exploratory in nature, we did not compare its antiplatelet activity with a standard antiplatelet drug like aspirin.

This study has few limitations. Ivy method even though more invasive, when used with a template is more reproducible than Duke method [12]. But compliance of study subject is a matter to be addressed as the incision in Ivy method is 10mm long and 1mm deep made on forearm. Duke method is not very sensitive test to confirm antiplatelet aggregatory effect. If it was possible we would have added another sensitive test for the assessment of the same. The gold standard for platelet aggregation test is Light Transmission Aggregometry (LTA) [21]. This instrument is available only in few institutes. Other methods are optical density method, impedance aggregometry also called Whole Blood Aggregometry (WBA), using a specialized instrument called Platelet Function Assay (PFA-100) and highly sensitive particle counting method with laser light scattering are also employed [16,21]. In laser light scattering technique it is possible to quantitate platelet aggregates and also to determine their sizes [16]. More studies with larger sample size using various doses of ARBs will help to ascertain its clinical implications and drug interactions with other antiplatelet agents. Furthermore as ARBs show antiplatelet activity, selectively prescribing these agents in high risk hypertensive patients could be beneficial.

CONCLUSION

ARB have antiplatelet activity. Increase in BT in ARB group when compared with that of control group is a reflection of antiplatelet activity. Antiplatelet aggregatory, along with antihypertensive effect of these agents could prove to be desirable in hypertensives with high atherosclerotic and thromboembolic risk.

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