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Research Article

Comparison of bleeding time changes in essential hypertension patients on losartan or amlodipine: a prospective observational study

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ABSTRACT

Background: Hypertension leads to vascular damage due to high pressure exerted on arteriolar wall and also promotes atherothrombosis in large and medium sized blood vessels. Thrombosis is an extension of haemostasis and platelets have a crucial role in the formation of atherothrombosis. Increased platelet activity is a risk factor in hypertensive patients and leads to cardio- and cerebrovascular events and target organ damage. Anti-platelet aggregatory treatment in these high risk patients have become a crucial step in their treatment. Recent data indicate that angiotensin II type 1 blockers or AT1 receptor blockers (ARBs) like Losartan and dihydropyridine class of L-type calcium channel antagonist like Amlodipine have anti- platelet activity. These two classes of drugs are frequently administered in hypertensive either alone and in combinations. This study aims to compare the anti-platelet activity of Losartan and Amlodipine. Anti-platelet activity in addition to anti-hypertensive activity of these drugs would be beneficial in treating hypertensive who are at high risk of atherosclerosis and atherothrombosis, if they are selectively prescribed these agents.

Methods: This was an observational study. Sixty (n=60) patients diagnosed with essential hypertension, attending medicine outpatient department of a tertiary care hospital were enrolled in the study. Out of them thirty (n=30) were patients who were prescribed losartan. Rest of the patients (n=30) were ones prescribed amlodipine. It was ensured that the patients of both the groups were on respective medication for at least one month. Another thirty (n=30) normotensive subjects acted as control. The bleeding time was evaluated for all three groups using Duke method of bleeding time estimation.

Results: Data was analysed using SPSS software version 20. One way ANOVA was used to analyse the data. This was followed by post hoc Tukey's test. The mean bleeding time(in minutes) of Losartan group was 2.583±0.263 SD, Amlodipine group was 2.214±0155 SD and control group was 1.998±0.198 SD. Statistically significant p value of <0.001 was observed in losartan and amlodipine groups.

Conclusions: Our study shows that the mean bleeding time of Losartan group and amlodipine group were significantly higher than that of Control group. It was further observed that the mean bleeding time of losartan was higher than that of amlodipine group indicating a better antiplatelet action by losartan than amlodipine. Additional antiplatelet activity could be desirable to treat hypertensive patients with high atherothrombotic and/or thromboembolic risk.

Keywords: Losartan, Amlodipine, Hypertension, Bleeding time, Antiplatelet activity

INTRODUCTION

Essential hypertension is associated with an increased risk of arterial thrombosis. Increased platelet activity is a significant contributor of this phenomenon and is due to various mechanisms like: endothelial dysfunction resulting from its denudation, overactive sympathetic and renin-angiotensin aldosterone systems, decreased platelet nitric oxide synthesis, and due to platelet degranulation occurring secondary to increased shear. 1,2 Nitric oxide (NO) is synthesized from L-arginine by the enzymes known as nitric oxide synthases (NOS). The 3 isoforms of NOS are: NOS1 [neuronal NOS (nNOS)], NOS2 [inducible NOS (iNOS)] and NO3 [endothelial NOS (eNOS)]. NOS1 and NOS3 are always expressed in cells and their enzymatic activity is regulated by intracellular Ca2+. NOS2 is not present in resting cells and presence of inflammatory stimuli is required to induce it. Its activity is Ca2+ independent. It has been found that NOS2 and NOS3 are expressed in platelets.3 Physiologically, in an intact vasculature, circulating platelets are maintained in an inactive state by NO and prostacyclin (PGI2) released by endothelial cells lining the blood vessels.⁴ NO has a significant role in the regulating vascular tone and in the controlling blood pressure. Various studies point out that reduction in normal basal NO release may predispose to hypertension, thrombosis, atherosclerosis and vasospasm. It has been found that Inhibition of eNOS elevates blood pressure in healthy humans and in mice disruption of eNOS gene leads to hypertension.⁵ In hypertension, shear due to increased pressure can cause endothelial damage as the mechanisms of normal vascular remodeling is faulty due to impaired structural adaptation to elevated blood pressure and vasoconstriction.^{6,7} When the vessel wall is damaged, sub endothelial matrix is exposed and platelets adhere to collagen with the help of $\alpha 2\beta 1$ and glycoprotein(GP)V1 and to Von Williebrand factor (vWF) with the help of $GPIb\alpha$ and GPIIb/IIIa present on the platelet surface. Adherent platelets secrete ADP from their dense granules, and synthesize and release thromboxane A2 (TXA2). ADP and TXA2 are platelet agonists and activate more platelets to the site of vascular injury. Activated platelets also potentiate coagulation by binding to clotting factors that lead to thrombin generation. Thrombin converts fibringen to fibrin and is also a potent platelet agonist. Hence platelets have an important role in formation of atherothrombosis in hypertensives and contribute to cardio-and cerebrovascular events leading to target organ damage.^{6,8} Anti-platelet treatment in hypertensives with high risk of thromboembolic and/or atherosclerosis is an established step in their treatment.9 Recent data indicate that angiotensin II type 1 blockers or AT1 receptor blockers (ARBs) like losartan and dihydropyridine class of L-type calcium channel antagonist like Amlodipine have antiplatelet activity. 10-12 These two classes of drugs are frequently administered in hypertensive either alone and in combinations. 8,13 Anti-platelet activity in addition to anti-hypertensive activity of these drugs would be beneficial in treating hypertensive who are at high risk of atherosclerosis and atherothrombosis, if they are selectively prescribed these agents. The present study aims to compare the antiplatelet activity of these two drugs in patients with essential hypertension. Bleeding time is a laboratory test that can be used to assess platelet function. So an increase in bleeding time can be a function of reduced platelet activity possibly by antiaggregatory action. Duke method of bleeding time estimation was used in this study. The present study are at high risk of atheroscients and atherothrombosis, if they are selectively present study are at high risk of atheroscients.

METHODS

It was an observational study. A total of (n=60) patients diagnosed with essential hypertension attending Medicine outpatient department of a tertiary care hospital were enrolled in the study. Among them group I consisted of thirty (n=30) patients who were prescribed Losartan. Rest of the thirty (n=30) in group II were on Amlodipine. Thirty (n=30) normotensive subjects taken as control and belonged to Group III. Initial baseline characteristics like SBP, DBP, age, sex, duration of treatment, average dose of drug used, average duration of treatment were recorded. Detailed written informed consent was taken from all study subjects. Subjects included in study were ≥ 18 years of age and of both sexes. Losartan and Amlodipine groups only included patients who received their respective drug for at least a period of one month. Patients with secondary hypertension, suffering from fever, comorbid bleeding disorders, any medication altering platelet function like aspirin, dipyridamole, statins etc., pregnant and lactating women were excluded from the study. Duke method of Bleeding time estimation was used to assess changes in Bleeding time in all the groups. 14,15 One Staff in the lab was trained for performing the test and recorded all the results. Lancet was used to prick left fingertip and a filter paper was used to wipe the blood every 15 seconds till the bleeding stops. Results were simultaneously observed and recorded by one investigator and on finding a difference in observed value mean was taken as the bleeding time.

Statistical analysis

All the data was expressed as mean±SD. One way ANOVA was used to compare the observed bleeding time of all groups. This was followed by post hoc Tukey's test. A p value of <0.05 was considered statistically significant. Statistical calculations were performed using SPSS Software version 20.

RESULTS

The baseline characteristics are represented in Table 1. As shown in Table 2, the mean bleeding time(in minutes) of Losartan group was 2.583±0.263 SD, Amlodipine group was 2.214±0155 SD and control group was 1.998±0.198 SD. Statistically significant p value of <0.001 was observed in all the groups. However, the mean bleeding time of losartan group (group I) 2.583±0.048 SD was

more than that of amlodipine group (group II) 2.214 ± 0.028 SD. The difference in bleeding time was statistically significant with a p value of < 0.001. Figure 1 shows the bar diagram representing the changes in mean bleeding time with SD of all the groups.

Table 1: Baseline characteristics of subjects.

	Group I (losartan)	Group II (amlodipine)	Group III (control)
Age	53.067	54.8	51.933
(in years)			
Sex			
Males	17	16	15
Females	13	14	15
SBP	129.667	130	121.667
DBP	86.667	84.667	83.333
Average	28.33	6.66	-
dose			
(mg/day)			
Average	2.75	3.683	-
Duration of			
treatment			
(in years)			

Table 2: Mean bleeding time (BT)±SD of losartan, amlodipine and control group.

	Mean BT(in minutes)	SD
Group I (losartan)*	2.583	0.263
Group II (amlodipine)* Group III (control)	2.214 1.998	0.155 0.198

^{*}Statistically significant p value of <0.001.

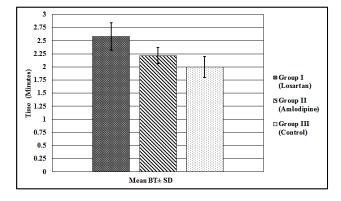


Figure 1: Bar diagram representing BT changes of losartan, amlodipine and control group.

DISCUSSION

Our study shows that the mean bleeding time of Losartan group was higher than that of Amlodipine indicating a better antiplatelet action by losartan. Angiotensin II type 1 antagonist or angiotensin receptor blockers (ARBs) and calcium channel blockers are commonly prescribed in hypertension. European society of hypertension and

european society of cardiology (ESH/ESC) 2013 guidelines recommend the use of these agents.¹³ In addition to lowering blood pressure they are known to have antiplatelet activity. Amlodipine belongs to dihydropyridine class of L-type calcium channel blockers. Platelets do not have L-type calcium channels but these agents exhibit antiplatelet activity nonetheless. Exact mechanism of action is not completely elucidated. Activation of NO/cGMP- dependant signalling pathway is one of the proposed mechanisms of anti-platelet activity. 12 Amlodipine can cause release of endothelium derived NO. It is found that amlodipine can phosphorylate eNOS through protein kinase C (PKC) pathway and enhance its functions. 16 NO thus formed binds to soluble guanylyl cyclase (sGC) which is a receptor for NO and converts GTP to cGMP.¹⁷ cGMP acts through cyclic GMP dependant protein kinase 1β (PKG1β). PKG1β is predominantly present in platelets. 12,18 NO mediated increase in cGMP inhibits platelet aggregation. 19 PKG1 promotes increased storage of Ca2+ in sarcoplasmic reticulum and inhibits inositol 1,4,5-tris phosphate stimulated Ca2+ release from sarcoplasmic reticulum. This results in decreased intracellular Ca2+ levels in platelets. Calcium is required for platelet activation and function. Decrease in intracellular Ca2+ in turn decreases platelet activation. PKG also inhibits TXA2 by phosphorylation. 12,18

Another study suggests antiplatelet activity of Amlodipine through PPAR β agonistic activity. Even though platelets are unucleated, they contain transcription factors like peroxisome proliferator-activated receptor (PPAR). Activation of PPARs inhibited platelet activation through a non-genomic mechanism and also increases NOS expression.

Inhibition of human platelet thromboxane A2/Prostaglandin H2 is the possible mechanism of action of losartan. Thromboxane A2 is a potent inducer of platelet aggregation. Physiologically platelet cAMP levels are regulated by TXA2 and PGI2. Increased intracellular concentration of cAMP in platelets activates protein kinase. A which through incompletely elucidated mechanism causes a decrease in intracellular calcium in platelets which in turn decrease platelet aggregability. The exact mechanism by which increased cAMP leads to decreased platelet aggregation is unknown as of yet.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. 10

CONCLUSIONS

Our study shows that the mean bleeding time of losartan group and amlodipine group were significantly higher than that of control group. It was further observed that the mean bleeding time of losartan was higher than that of amlodipine group indicating a better antiplatelet action by losartan than amlodipine. Additional antiplatelet activity could be desirable to treat hypertensive patients with high atherothrombotic and/or thromboembolic risk.

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Institutional Ethics Committee

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