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EFFECT OF LOSARTAN ON HEMOSTATIC PROFILE IN RATS

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Abstract: Ace inhibitors like ramipril have been shown to affect coagulation profile in rats. This study was carried out to find the interactions between aspirin and losartan on coagulation profile. Three doses of losartan and its combination with aspirin were tested for their effect on hemostatic profile in albino Wistar rats. The findings of the study suggest that there is a possibility of interaction between aspirin and losartan when used together in patients.

Key Words: Angiotensin receptor blocker, Losartan, rats, INR.

INTRODUCTION

Angiotensin receptor blockers (ARBs) are used in cardiovascular disorders such as hypertension and acute myocardial infarction, often along with aspirin, whenever patients do not tolerate angiotensin converting enzyme inhibitors (ACEIs). Pronounced prolongation in bleeding time & prothrombin time by the tissue angiotensin converting enzyme inhibitor (ACEI) ramipril is demonstrated, in normal rats.[1] As angiotensin receptor blockers (ARBs) also inhibit the renin angiotensin pathway, they also are likely to have similar effects. They are likely to be combined with aspirin in the treatment of cardiovascular diseases like heart failure and myocardial infarction. Though the synergistic interaction between these two drugs could be beneficial in cardiovascular diseases, physicians also have to be careful about the possible adverse effect on coagulation profile. As angiotensin converting enzyme inhibitors (ACEIs) have been shown to affect arterial thrombosis it will be important to know the effects of angiotensin blockers (ARBs) like losartan on coagulation profile. As no data is available it was decided to evaluate the effects of losartan alone and in combination with aspirin on hemostatic profile in rats.

MATERIALS AND METHODS

Animals

The study was carried out after obtaining permission from institutional animal ethics committee. Albino Wistar rats weighing between 200-250 g were used for the study. They were kept in polypropylene cages in air conditioned laboratory where 12 hours light-dark cycle was maintained. Animals were provided with normal rat food and water ad-libitum. They were divided into 24 experimental groups (Eight groups each for estimating clotting time, bleeding time and International normalized ratio) of six animals each. Various groups for each of the experiment were- Control (normal saline), aspirin (standard drug), three groups for three doses of losartan (5mg/kg, 10mg/kg and 20mg/kg), and three groups for combination of losartan and aspirin (Losartan 5mg/kg + Aspirin 5mg/kg, Losartan 10mg/kg + Aspirin 5mg/kg and Losartan 20mg/kg + Aspirin 5mg/kg). [Table 1 & 2]

Drugs

Control group of rats were administered normal saline. Losartan was administered in three doses [5, 10 and 20mg/kg] alone and in combination with aspirin [5mg/kg], in different experimental groups for 10 days. Aspirin [5mg/kg] alone was administered in one group of animals in each experimental group for 10 days. All doses were given orally using gavage.

Estimation of bleeding time

The bleeding time for all animals was done at baseline (day 0) & post treatment on day 11. The bleeding time estimation was done by the saphenous vein puncture. [2]

Estimation of clotting time[3, 4]

Clotting time estimation was done by the retro-orbital sinus puncture and capillary glass method.

Prothrombin time

The blood sample required for prothrombin time estimation was acquired through intracardiac blood collection.[5] Cascade® 480 was used for estimation of prothrombin time by photo-optical method in the biochemical laboratory and the results expressed in international normalized ratio [INR]. [6]

Statistical analysis

One way ANOVA followed by Tukey's post-hoc test was applied using statistical package, Graphpad v 3.1.

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RESULTS

Bleeding time

Differences in bleeding time at baseline among different groups were statistically not significant. There was prolongation of bleeding time when losartan was administered alone in a dose of 20mg. /kg. However in lower doses (10mg and 20mg/kg) losartan did not show any effect on bleeding time, compared to control group. Bleeding time was significantly ($p<0.05$) prolonged, compared to control group, among the groups of rats which were treated by aspirin and combination aspirin and three different doses of losartan. (Table 1)

Table 1: Bleeding time of rats in seconds (Mean±SD) at baseline and post treatment

GROUPS (n=6)	Baseline Day 0	Post treatment Day 11
Control	70±14.31	71±12.32
Aspirin 5mg/kg	80±15.49	180±13.66*
Losartan 5mg/kg	70±15.49	69±10.34
Losartan 10mg/kg	70±15.49	71±12.1
Losartan 20mg/kg	75±16.43	150±18.97*
Losartan 5mg/kg + Aspirin 5mg/kg	70±15.49	175±12.24*
Losartan 10mg/kg + Aspirin 5mg/kg	70±15.49	172±11.78*
Losartan 20mg/kg + Aspirin 5mg/kg	65±12.24	187±18.97*

*p<0.05, Compared to control.

International normalized ratio (INR)

INR was prolonged ($p<0.05$) among all the groups when compared to control group except among rats, which were administered lower doses of losartan (5mg/kg and 10mg/kg). When losartan was administered alone, only the dose of 20mg/kg showed significant prolongation of INR compared to aspirin ($p<0.05$). When losartan was administered along with aspirin, only the group where losartan was used in 20mg/kg along with aspirin showed statistically significant prolongation in INR compared to aspirin. ($p<0.05$). Prolongation of INR among other two groups was comparable to that with aspirin group.

Clotting time

None of the drugs had any effect on clotting time.

DISCUSSION

A study carried out by HN Gopalakrishna et al has shown that ACEIs prolong bleeding time and INR. However lower doses of ACEIs did not prolong bleeding time or INR in their study.[2] The likely mechanism could be the inhibition of synthesis of angiotensin II in the endothelium and attenuation of prothrombotic effect. Second mechanism could be an increase in bradykinin concentration which enhances the release of NO, PGI_2 & t-PA which are strong antithrombotic agents. [7, 8]

Table 2: INR of rats in seconds (Mean±SD) at baseline and post treatment

GROUPS (n=6)	Baseline Day 0	Post treatment Day 11
Control	0.94±0.053	0.938±0.153
Aspirin 5mg/kg	0.905±0.057	1.4±0.132*
Losartan 5mg/kg	0.983±0.065	0.991±0.192
Losartan 10mg/kg	0.958±0.064	0.936±0.155
Losartan 20mg/kg	0.948±0.045	2.196±0.177*
Losartan 5mg/kg + Aspirin 5mg/kg	0.983±0.065	1.3± 0.128#*
Losartan 10mg/kg + Aspirin 5mg/kg	0.958±0.064	1.5± 0.122#*
Losartan 20mg/kg + Aspirin 5mg/kg	0.948±0.045	3.667± 0.159*$

*P<0.05, compared to control. #P>0.05, compared to aspirin. $P<0.05 compared to aspirin

In the present study, prolongation of INR and bleeding time with losartan alone was dose related and it was statistically significant only at the highest dose (20mg/kg). Prolongation of INR at the highest dose was much higher than that produced by aspirin (5mg/kg). Prolongation of INR has increased with increase in the dose. Prolongation of INR at higher doses was more than that seen with aspirin. It has been previously demonstrated that the AT_1–antagonist losartan exerts strong anti-thrombotic effect. It is a competitive antagonist of the thromboxane A_2 receptor and attenuates platelet aggregation. [9]

CONCLUSION

The findings of the study suggest that there is a possibility of interaction between aspirin and losartan when used together in patients. This is more important at higher doses of Losartan. Normally such interaction would have beneficial antithrombotic effect at least in lower doses of losartan. However adverse events of unexpected bleeding should be borne in mind by the physicians whenever ARBs are combined with aspirin in patients.

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