

The Effects of Eprosartan on Cytoplasmic Free Calcium Mobilisation, Platelet Activation and Microparticle Formation in Hypertension. Could they be Relevant to Stroke Prevention?

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Abstract

The study examined a number of parameters of platelet function and activation in hypertensive patients compared with normotensive patients. It also examined the effects of the angiotensin receptor blocker eprosartan on these parameters. There were 30 patients with stage 1 or stage 2 hypertension plus 31 well-matched controls in this study.

Phosphatidylserine, a measure of circulating activated platelets, was expressed more in hypertensive patients than in controls. Eprosartan partially corrected the enhanced platelet reactivity. There were greater numbers of activated platelet microparticles in hypertensive patients compared to controls. Again, eprosartan reduced this hyperproduction.

Calcium kinetics of platelets were measured with flow cytometry using fluorescent markers. The free calcium concentration and its rise in response to thrombin were greater in hypertensive patients. Eprosartan significantly changed these parameters towards control values and reduced platelet activation.

Thus, eprosartan has the potential to reduce the risk of atherosclerotic events in hypertensive patients.

Introduction

Arterial hypertension is the main risk factor for atherothrombotic disease of the heart and brain among adults. Activation of the renin-angiotensin-aldosterone system (RAAS) contributes to structural changes in the blood vessels and cell proliferation. It is also involved in regulation of coagulation and fibrinolysis, including platelet function. Thus, blockade of the RAAS using agents such as angiotensin receptor blockers (ARBs) could have an influence on the abnormalities such as these that are observed in hypertension. Platelets themselves have type 1 receptors for angiotensin II that, when activated, cause opening of calcium channels. Platelets from the hypertensive rat have been shown to exhibit high calcium levels in the resting state, possibly due to enhanced calcium entry and mobilisation. ARBs have also been shown to modify favourably levels of factors such as plasminogen activator

inhibitor-1 (PAI-1), fibrinogen and thrombomodulin.

In this study platelet function in hypertensive patients was examined, at baseline and after treatment with eprosartan, and compared with controls. The objectives of the study were:

- To establish whether hypertensive patients have higher platelet activation compared to normotensive patients. If so, could higher platelet activation be caused by greater numbers of circulating activated platelets or by their increased responsiveness to agonists
- To establish whether the ARB eprosartan has effects on platelet function in addition to its antihypertensive effects
- To determine whether any changes in platelet activation might be achieved by alterations in calcium kinetics.

Study Methodology

The patients consisted of 30 (15 male and 15 female) patients with stage 1 or 2 hypertension according to World Health Organization criteria. Their mean age was 47.6 ± 9.4 years and their mean body mass index was 27.9 ± 3.9 kg/m². They had no renal, cardiac or hepatic lesions or diabetes. The patients were compared with 31 well-matched controls (14 male, 17 female), and all subjects continued with their ordinary diet and lifestyle. The hypertensive patients were evaluated at baseline after a two-week wash out period and then after one and two months' treatment with eprosartan 600 mg once daily.

A number of parameters of platelet function were evaluated using flow cytometry techniques, using whole blood withdrawn from an antecubital vein after a 12-hour fast. The first parameter, phosphatidylserine (PS), is a measure of circulating activated platelets: when exposed on the external hemilayer of the platelet membrane, it forms the prothrombinase complex, which is a risk factor for a hypercoagulable state. To assess PS, the percentage of CD61- and annexin V-positive events were recorded in the gate corresponding to platelets. Second, activated platelet microparticles (PMP), also demonstrate prothrombinase activity

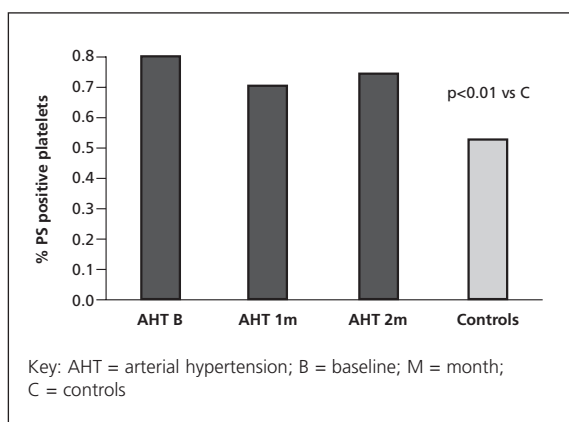


Figure 1
Effect of eprosartan on PS expression *in vivo*

and therefore may contribute to the development of atherothrombosis. To assess these, the number of CD42b- and annexin V-positive events were identified in the gate below the zone of the platelets.

Third, calcium mobilisation was measured, because different steps of platelet activation are accompanied by changes in free cytoplasmic calcium concentration. In hypertension, elevations in cytosolic calcium are related to enhanced vascular tone and a tendency towards platelet activation. To assess calcium mobilisation in this study, fluo-3-AM fluorescence intensity was measured before and after addition of 0.1 U/mL thrombin, and the slope of secondary fluorescence decrease or normalisation was calculated.

Results

Blood Pressure

As anticipated, administration of eprosartan tended to normalise the blood pressure, with significant differences compared to baseline values after one and two months of treatment.

Phosphatidylserine

Figure 1 shows the effects of eprosartan on PS expression *in vivo*. Hypertensive patients expressed more PS than controls ($p<0.01$). Shear stress, even when applied in low, physiological amounts, suffices *in vitro* to induce platelet activation in both hypertensive and normotensive patients, and this increased activation is reduced by eprosartan treatment. At baseline, platelet reactivity in response to calcium ionophore *in vitro* was higher among hypertensive patients than among controls. After two months of treatment with eprosartan, this enhanced reactivity was partially corrected ($p<0.05$ compared to baseline).

Platelet Microparticles

Figure 2 shows the effects of eprosartan on microparticle formation *in vivo*. Hypertensive patients have more circulating microparticles

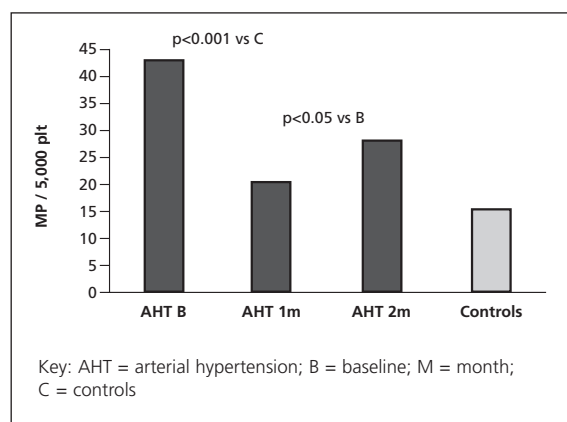


Figure 2
Effect of eprosartan on microparticle formation *in vivo*

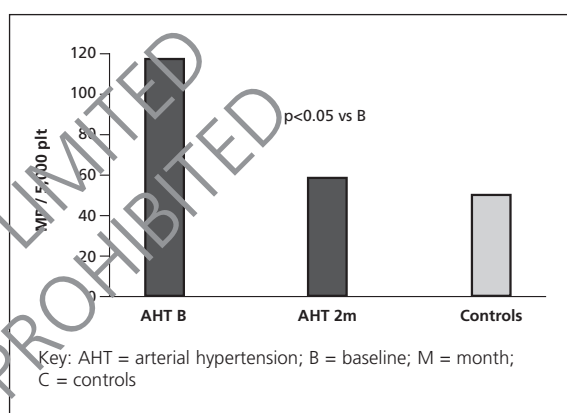


Figure 3
Effect of eprosartan on microparticle generation by shear stress (230 s^{-1} , 5 min)

than controls (44/5,000 *vs.* 15/5,000 platelets, respectively, $p<0.001$) and eprosartan treatment reduces this hyperproduction ($p<0.05$ compared to baseline). Again, hypertensive patients show a higher number of microparticles after shear stress and calcium ionophore exposure.

Figure 3 shows the effect of eprosartan on microparticle generation in response to shear stress. Microparticle generation is much higher in hypertensive patients at baseline. This enhanced microparticle generation is brought approximately to the level seen in controls after eprosartan treatment. It is possible that the effects of eprosartan seen here may represent additional benefits for the drug and may prevent thrombotic events.

Calcium Kinetics

Flow cytometry using fluorescent markers was used to evaluate platelets in three principal ways. The first measure is basal fluorescence, which is related to the free calcium concentration (and tended to be higher in the hypertensive patients). Second is the rise in fluorescence after administration of thrombin, which is a measure of platelet responsiveness. Third is the slope of dis-

Table 1 Effect of eprosartan on cytoplasmic free calcium thrombin-induced mobilisation		
	Initial FAU	Increase Thr 0.1 u/mL
AHT baseline	3.00±6.19	269±1.44**
1 m Epro	3.25±7.55	247±123
2 m Epro	0.74±2.12†	165±33.6††
Controls	2.35±4.11	247±90

**p<0.01 vs C; †p<0.05 vs B; ††p<0.001 vs B
Key: FAU = fluorescence arbitrary units; AHT = arterial hypertension

Table 2 Intraplatelet free calcium decrease gradient in native blood at baseline and after 1 and 2 months of eprosartan therapy	
	m Native blood
AHT baseline	-33.6±14.9***
1 m Epro	-35.2±10.1
2 m Epro	-37.8±5.6††
Controls	-37.9±5.9

*** p<0.001 vs C; ††p<0.01 vs B
Key: AHT = arterial hypertension

appearance of fluorescence, or normalisation: the greater the slope, the greater the tendency towards normalisation.

Table 1 shows the effects of eprosartan on cytoplasmic free calcium thrombin-induced mobilisation. There is a higher concentration initially in hypertensive patients, and the fluorescence increment induced by thrombin (which must be related to the increase in calcium concentration) was greater among hypertensive patients. As can be seen from the table, eprosartan significantly alters both these parameters towards control values and reduces platelet activation.

Table 2 shows the decrease in gradient of intraplatelet free calcium, at baseline and after eprosartan treatment. It can be seen that the slope normalises less quickly in hypertensive patients at baseline, suggesting that the platelets of hypertensive patients remain active for a longer period.

Conclusions

In addition to the effects of eprosartan on reducing elevated blood pressure (which itself has haemodynamic and other benefits), it seems able to block the angiotensin II receptors type 1 on platelets. Thus, eprosartan effectively controls and normalises alterations in platelet function, probably through modifying calcium mobilisation. This has the potential to reduce the risk of atherothrombotic events in hypertensive patients and may explain the beneficial effects of this drug in stroke reduction that were observed in the Morbidity and mortality after Stroke – Eprosartan compared with nitrendipine for Secondary prevention (MOSES) trial (see the article reporting the presentation by Dr Lüders on page S12-S15 of this supplement).

References

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