ORIGINAL ARTICLE

Impact of the therapy by renin–angiotensin system targeting antihypertensive agents perindopril versus telmisartan on prothrombotic state in essential hypertension

A Remková, H Kratochvíl’ová and J Šturina
Department of Internal Medicine, School of Medicine, Comenius University in Bratislava, Bratislava, Slovak Republic

The aim of the study was to investigate the effect of therapy by perindopril or telmisartan on endothelial/platelet function and on coagulation/fibrinolysis in 20 and 16 hypertensive patients, respectively. The measurements were carried out before and after 1 month of therapy. Both systolic blood pressure and diastolic blood pressure were reduced ($P<0.001$) or normalized due to each therapy. Plasma thrombomodulin (TM) and von Willebrand factor (vWF) as indicators of endothelial dysfunction, plasma $\beta$-thromboglobulin ($\beta$-TG), platelet factor 4 (PF4), soluble P-selectin (sPsel) and soluble glycoprotein V (sGpV) as indicators of in vivo platelet activation, plasminogen activator inhibitor type 1 (PAI-1) antigen and tissue type plasminogen activator (tPA) antigen as markers of fibrinolytic activity, plasminogen activator inhibitor type 1 (PAI-1) antigen and tissue type plasminogen activator (tPA) antigen as markers of fibrinolytic activity, soluble endothelial protein C receptor (sEPCR) as a new marker of hypercoagulation and fibrinogen level as a known risk factor for vascular changes were investigated. A decrease of plasma vWF, sPsel, sGpV, PAI-1 and tPA antigen level ($P<0.05$, respectively) after 1 month of therapy by perindopril was observed. On the other hand, a decrease of plasma sEPCR and fibrinogen level ($P<0.05$, respectively) after 1 month of therapy by telmisartan was found. We failed to find changes of plasma TM, $\beta$-TG and PF4 due to any therapy investigated. The additional beneficial ‘antithrombotic’ effects of the renin–angiotensin system targeting agents (vasculoprotective, anti-platelet and profibrinolytic effects of perindopril and anticoagulant/rheological effects of telmisartan) may be important in terms of the favourable role of antihypertensive drugs in cardiovascular morbidity.

Keywords: perindopril; telmisartan; endothelial/platelet function; fibrinogen; fibrinolysis

Introduction

Hypertension is a major risk factor for thrombotic events such as myocardial infarction and stroke, reflecting a prothrombotic state that is present in hypertensive patients. A number of rheological, haemostatic, endothelial and platelet abnormalities appear to play a role in the thrombotic complications of hypertension. This prothrombotic/hypercoagulable state in hypertension may contribute to the increased risk and severity of target organ damage.1–3 Moreover, markers of a hypercoagulable state may predict subsequent cardiovascular events in hypertensive patients.4

A growing body of evidence indicates that prothrombotic state can be induced by the activated renin–angiotensin system (RAS), which is more pronounced in hypertension. The RAS plays an important role in the pathogenesis of atherosclerotic complications. It can influence not only vascular tone, but also disturb the balance of the haemostatic system, with abnormalities in endothelial and platelet function, coagulation and fibrinolysis.5,6 Therefore, it is of importance to regulate not only blood pressure (BP), but also the haemostatic system in the long-term antihypertensive therapy. From this point of view, treatment of uncomplicated essential hypertension by RAS inhibition-based antihypertensive therapy could result in a reversal of prothrombotic abnormalities, contributing to a reduction in thrombosis-related complications.
Evidence for the protective role of some RAS targeting agents against atherothrombotic cardiovascular disease is accumulating. A precise mechanism of their ability to prevent thrombotic events is of particular interest. Both angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT₁) receptor blockers (ARB) effectively decrease BP, but it seems that there could be some differences in clinical outcome, which may be partly related to their effect on haemostatic abnormalities.

Both the studied drugs, that is ACE inhibitor perindopril and the ARB telmisartan target the RAS in hypertension and they fulfil all the requirements of modern antihypertensive drugs. Perindopril is the prodrug that is transformed through de-esterification into perindoprilat, a potent ACE inhibitor. This agent has a high vascular affinity and binds both to plasma and tissue ACE—especially in the vascular endothelium and adventitia. Now, a new form of perindopril, that is perindopril arginine is available which can be expected to be more advantageous. Telmisartan, a highly lipophilic ARB (but not other AT₁ receptor antagonists) has been shown to act as a partial agonist of the peroxisome proliferator-activated receptor-γ, which plays a role in the regulation of carbohydrate and lipid metabolism.

The aim of the present study was to investigate the effect of 1-month therapy by perindopril or telmisartan, on endothelial and platelet function, coagulation/fibrinolysis in patients with the early stages of essential hypertension. Since these drugs have two distinct mechanisms of RAS interruption, it is hypothesized that each therapy might have different impact on the prothrombotic state in hypertensive patients, focusing on some parameters of haemostasis. We tested the hypothesis that perindopril or telmisartan therapy reduced endothelial and platelet markers and improved coagulation/fibrinolysis over a period of 1 month. The values of these markers after therapy were compared with those before therapy in the same patients.

### Materials and methods

#### Subjects

Thirty-six patients (white Caucasian), 25 women and 11 men, aged from 26 to 83 years (mean 55.8 ± 14.2 years) with untreated mild-to-moderate essential hypertension, were taken into this study. Despite a left ventricular hypertrophy in about one-third of hypertensive patients, they were free of other target organ damage. Moreover, a population of 26 healthy normotensive subjects (non-smokers) of similar sex (15 women and 11 men), age (from 33 to 75 years, mean 51.8 ± 10.5 years) and ethnic origin for assessment of normal values of the laboratory markers (Tables 1 and 2) was also examined. None of these healthy subjects were taking any medication. Their systolic BP (SBP) was 123.3 ± 8.9 mmHg and diastolic BP (DBP) 74.8 ± 5.0 mmHg.

Arterial hypertension was defined as SBP ≥ 140 and/or DBP ≥ 90 mmHg on at least three occasions. The diagnosis of essential hypertension was made by physical examination, routine and special laboratory tests (including microalbuminuria, serum chromogranin and other endocrine tests), echocardiography and radiologic investigations. We selected only patients with normal 24-h urine excretion of albumin (<25 mg per day) who had normal cardiac function as measured by echocardiography. Their body mass index was 27.3 ± 2.0 kg m⁻². Patients with evidence of manifest vascular diseases, secondary hypertension, kidney or heart failure, diabetes mellitus, liver disease, neoplastic or inflammatory disease and smokers were excluded. A role of inflammation was ruled out.

### Table 1 The baseline clinical characteristics of the healthy subjects and all the patients with hypertension investigated

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (n = 26)</th>
<th>Hypertensive patients (n = 36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.8 ± 10.5</td>
<td>55.8 ± 14.2</td>
<td>0.23</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>11/15</td>
<td>11/25</td>
<td>0.15</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123.3 ± 8.9</td>
<td>148.4 ± 12.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.8 ± 5.0</td>
<td>91.9 ± 7.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>25.2 ± 1.6</td>
<td>27.3 ± 2.0</td>
<td>0.44</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>83 ± 4</td>
<td>85 ± 6</td>
<td>0.56</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol l⁻¹)</td>
<td>5.0 ± 0.3</td>
<td>5.4 ± 0.4</td>
<td>0.47</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol l⁻¹)</td>
<td>4.5 ± 0.5</td>
<td>5.8 ± 1.3</td>
<td>0.039</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol l⁻¹)</td>
<td>3.4 ± 0.5</td>
<td>4.3 ± 0.6</td>
<td>0.041</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol l⁻¹)</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Serum triglyceride (mmol l⁻¹)</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>0.38</td>
</tr>
<tr>
<td>Serum creatinine (μmol l⁻¹)</td>
<td>65.5 ± 9.4</td>
<td>68.0 ± 10.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Platelet count (10⁹ per liter)</td>
<td>280 ± 64</td>
<td>302 ± 72</td>
<td>0.48</td>
</tr>
<tr>
<td>C-reactive protein (mg ml⁻¹)</td>
<td>1.66 ± 0.35</td>
<td>1.86 ± 0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>Chromogranin (ng ml⁻¹)</td>
<td>63.7 ± 22.2</td>
<td>92.0 ± 128.4</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Abbreviations: BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are means ± s.d., compared by Student’s unpaired t-test or χ²-test, as appropriate.
out by C-reactive protein determination (with values always less than 5 mg ml\(^{-1}\)). All patients with known or suspected thrombotic disorders, patients taking any medication that might affect the platelet functions, such as statins or any anti-platelet agents including aspirin or oral anticoagulants, were also excluded. In a case of any other therapy this was not changed during all the period of the study.

The study patients were randomized and divided into two groups; 20 patients (14 women and 6 men, mean 56.9 ± 14.6 years) with oral intake of perindopril arginine (Prestarium A, Servier, Neuilly-sur-Seine, France) 40 mg per day, and 16 patients (11 women and 5 men, mean 53.9 ± 13.9 years) with oral intake of telmisartan (Micardis, Boehringer Ingelheim, Ingelheim, Germany) 40 mg per day. The values of their BP before therapy (at the inclusion to the study) and after 1 month of antihypertensive therapy by perindopril or telmisartan are shown in Table 3. There were not any significant changes in baseline SBP or DBP and in other basic characteristics between these two drug-studied groups. Blood samples were collected from all study patients before therapy and after 1 month of drug administration.

The study protocol was in agreement with the guidelines approved by the ethical committee of our institution and all subjects had given informed consent.

**Laboratory methods**

In each of the subject laboratory markers of endothelial and platelet function, as well as markers of hypercoagulation and fibrinolytic activity were studied. Plasma thrombomodulin (TM) and von Willebrand factor (vWF) were used as indicators of endothelial dysfunction and/or damage. Plasma \(\beta\)-thromboglobulin (\(\beta\)TG), platelet factor 4 (PF4), soluble P-selectin (sPsel), soluble glycoprotein V

### Table 2

<table>
<thead>
<tr>
<th>Healthy subjects (n = 26)</th>
<th>Hypertensive patients (n = 36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM (ng ml(^{-1}))</td>
<td>29.2 ± 10.9</td>
<td>35.1 ± 14.6</td>
</tr>
<tr>
<td>vWF (IU per 100 ml)</td>
<td>68.3 ± 20.1</td>
<td>79.1 ± 23.4</td>
</tr>
<tr>
<td>(\beta)TG (IU ml(^{-1}))</td>
<td>64.8 ± 41.3</td>
<td>72.0 ± 30.5</td>
</tr>
<tr>
<td>PF4 (IU ml(^{-1}))</td>
<td>10.8 ± 5.2</td>
<td>12.2 ± 3.4</td>
</tr>
<tr>
<td>sPsel (ng ml(^{-1}))</td>
<td>31.2 ± 14.3</td>
<td>42.1 ± 18.4</td>
</tr>
<tr>
<td>sGpV (ng ml(^{-1}))</td>
<td>44.9 ± 9.8</td>
<td>54.3 ± 41.2</td>
</tr>
<tr>
<td>PAI-1 antigen (ng ml(^{-1}))</td>
<td>32.5 ± 26.5</td>
<td>46.2 ± 27.4</td>
</tr>
<tr>
<td>tPA antigen (ng ml(^{-1}))</td>
<td>8.7 ± 5.6</td>
<td>12.7 ± 4.3</td>
</tr>
<tr>
<td>sEPCR (ng ml(^{-1}))</td>
<td>80.4 (47.6–92.6)</td>
<td>94.6 (87.2–111.9)</td>
</tr>
<tr>
<td>Fibrinogen (g l(^{-1}))</td>
<td>2.95 ± 0.66</td>
<td>3.63 ± 0.76</td>
</tr>
</tbody>
</table>

**Abbreviations:** \(\beta\)TG, \(\beta\)-thromboglobulin; PAI-1, plasminogen activator inhibitor type 1; PF4, platelet factor 4; sEPCR, soluble endothelial protein C receptor; sGpV, soluble glycoprotein V; sPsel, soluble P-selectin; TM, thrombomodulin; tPA, tissue plasminogen activator; vWF, von Willebrand factor.

**P-values**—Student’s unpaired t-test (in normally distributed groups) or Mann–Whitney U-test (in non-normally distributed groups).

### Table 3

<table>
<thead>
<tr>
<th>Perindopril baseline</th>
<th>Follow-up</th>
<th>Telmisartan baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>149.0 ± 12.9</td>
<td>125.0 ± 5.0*</td>
<td>147.7 ± 11.7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>91.3 ± 7.2</td>
<td>78.1 ± 7.2*</td>
<td>92.7 ± 8.1</td>
</tr>
<tr>
<td>TM (ng ml(^{-1}))</td>
<td>37.6 ± 17.5</td>
<td>38.4 ± 10.6</td>
<td>31.2 ± 6.6</td>
</tr>
<tr>
<td>vWF (IU dl(^{-1}))</td>
<td>81.9 ± 22.1</td>
<td>72.9 ± 23.3**</td>
<td>75.5 ± 25.3</td>
</tr>
<tr>
<td>(\beta)TG (IU ml(^{-1}))</td>
<td>71.6 ± 41.6</td>
<td>72.9 ± 28.9</td>
<td>53.5 ± 39.9</td>
</tr>
<tr>
<td>PF4 (IU ml(^{-1}))</td>
<td>11.0 ± 5.4</td>
<td>10.2 ± 4.3</td>
<td>10.3 ± 5.1</td>
</tr>
<tr>
<td>sPsel (ng ml(^{-1}))</td>
<td>46.1 ± 19.4</td>
<td>40.8 ± 13.8**</td>
<td>38.3 ± 16.0</td>
</tr>
<tr>
<td>sGpV (ng ml(^{-1}))</td>
<td>71.0 ± 40.5</td>
<td>52.3 ± 15.0**</td>
<td>50.4 ± 16.6</td>
</tr>
<tr>
<td>PAI-1 antigen (ng ml(^{-1}))</td>
<td>54.0 ± 27.9</td>
<td>41.4 ± 23.4**</td>
<td>38.4 ± 23.1</td>
</tr>
<tr>
<td>tPA antigen (ng ml(^{-1}))</td>
<td>14.0 ± 5.0</td>
<td>12.4 ± 3.5**</td>
<td>11.0 ± 3.4</td>
</tr>
<tr>
<td>sEPCR (ng ml(^{-1}))</td>
<td>111.9 ± 59.5</td>
<td>105.7 ± 111.9</td>
<td>107.5 ± 38.3</td>
</tr>
<tr>
<td>Fibrinogen (g l(^{-1}))</td>
<td>3.6 ± 0.7</td>
<td>3.4 ± 0.8</td>
<td>3.7 ± 0.8</td>
</tr>
</tbody>
</table>

**Abbreviations:** \(\beta\)TG, \(\beta\)-thromboglobulin; DBP, diastolic blood pressure; PAI-1, plasminogen activator inhibitor type 1; PF4, platelet factor 4; sEPCR, soluble endothelial protein C receptor; sGpV, soluble glycoprotein V; sPsel, soluble P-selectin; TM, thrombomodulin; tPA, tissue plasminogen activator; vWF, von Willebrand factor.

*\(P<0.001*, **\(P<0.05\)—in comparison with the baseline values (compared by the Student’s paired t-test).
Commercially available Asserachrom vWF kit (Diagnostica Stago, Asnières, France). Plasma vWF antigen concentration was measured by an enzyme immunoassay using a commercially available Asserachrom vWF kit (Diagnostica Stago). Plasma βTG, PF4 and sGpV were assayed by a commercial enzyme immunoassay Asserachrom β-TG kit, Asserachrom PF4 kit and Asserachrom sGPV kit (Diagnostica Stago), respectively. Plasma sEPCR was determined by an enzyme immunoassay (R&D Systems, Minneapolis, MN, USA).

Plasma TM was determined by an enzyme immunoassay using a diagnostic Asserachrom Thrombomodulin kit (Diagnostica Stago, Asnières, France). Plasma vWF antigen concentration was measured by an enzyme immunoassay using a commercially available Asserachrom vWF kit (Diagnostica Stago). Plasma βTG, PF4 and sGpV were assayed by a commercial enzyme immunoassay Asserachrom β-TG kit, Asserachrom PF4 kit and Asserachrom sGPV kit (Diagnostica Stago), respectively. Plasma sEPCR was determined by an enzyme immunoassay (R&D Systems, Minneapolis, MN, USA).

The total circulating PAI-1, both free and in complex with tPA (in active and inactive forms), was measured as antigen by an enzyme immunoassay procedure using the Asserachrom PAI-1 kit (Diagnostica Stago). The Asserachrom tPA kit (Diagnostica Stago), an enzyme immunoassay procedure, was used for the determination of plasma tPA antigen concentration. Plasma sEPCR was determined by an enzyme immunoassay using Asserachrom sEPCR kit (Diagnostica Stago). Plasma fibrinogen level was determined by means of Turbox Fibrinogen Assay (Orion Diagnostica, Espoo, Finland), a liquid phase immunoprecipitation and quantitative end point detection with Turbox analyser (Orion Diagnostica).

All blood collections were carried out between 0800 and 0900 (before administration of the daily dose of the treatment) after an overnight fast and an 8-h supine rest. Blood was drawn with minimal trauma from the antecubital vein, without venous stasis. All measurements of BP and blood collections were made in the supine position, after the patient had rested for 10 min.

Blood for TM, vWF, sPsel, tPA, sEPCR and fibrinogen determination was collected using Monovette 9NC tubes of Sarstedt system (Sarstedt, Nümbrecht, Germany). Plasma was separated by centrifugation at 2500 g for 10 min (within 30 min of collection). A portion was immediately frozen at −70 °C until tested for the TM, vWF, sPsel, tPA and sEPCR level. The fibrinogen concentration was determined immediately after collection from the remaining sample.

Simultaneously, blood for βTG, PF4, sGpV and PAI-1 assay was collected from the same venepuncture into a 10 ml plastic disposable syringe. An aliquot (4.5 ml) of blood was immediately transferred to each of two pre-cooled tubes (Diatube-H, Becton Dickinson, Plymouth, UK), which are specially designed to prevent platelet activation, supplied by Becton Dickinson (Plymouth, UK). The content was gently stirred, and the tubes were placed back to the ice-cold water bath within 2 min from the beginning of blood collection. The tubes were allowed to cool in the ice-cold water bath for at least 15 min. Then the blood samples were centrifuged at 2500 g for 30 min at 4 °C within the hour. One-third of the supernatant volume from the middle region of the liquid portion with platelet-poor plasma was collected. In order to achieve maximal platelet removal, this mid-layer platelet-poor plasma was centrifuged by the same way a second time. One-third of the supernatant was collected as previously described. The separated plasma was frozen at −70 °C and stored in aliquots until analysed.

Statistical analysis

Normally distributed data, presented as mean ± standard deviation (s.d.) were analysed by Student’s t-test. Non-normally distributed data, presented as median and interquartile range were analysed by the non-parametric Mann–Whitney U-test. Student’s unpaired t-test or Mann–Whitney U-test was used for the evaluation of the differences in the values between the healthy subjects and hypertensive patients as well as between hypertensive patients treated with perindopril and telmisartan. The effect of perindopril or telmisartan therapy on BP and laboratory variables in hypertensive patients was evaluated by the Student’s paired t-test, as the differences between the groups were tested within the same subject. The differences in gender were calculated by χ²-test. Baseline values of laboratory variables were set as 100%, and percentage of their changes after perindopril or telmisartan therapy was calculated. A value of P≤0.05 was considered statistically significant. All analyses were performed with SPSS computer programme.

Results

The baseline clinical characteristics of healthy subjects and patients with hypertension are depicted in Table 1. There were no significant differences among all the study groups with respect to age, sex, obesity, glycaemia, high-density lipoprotein cholesterol, triglyceride, serum creatinine, platelet counts, C-reactive protein and chromogranin level. The value of SBP, DBP, total and low-density lipoprotein cholesterol was significantly higher in hypertensive patients compared with control group of healthy subjects. No significant changes in SBP, DBP and haemostasis parameters between hypertensive patients treated by perindopril or telmisartan neither before therapy nor after therapy were found.
In comparison with healthy subjects, a statistically significant increase of TM ($P = 0.05$), vWF ($P = 0.049$), sPsel ($P = 0.015$), sGpV ($P = 0.015$), PAI-1 antigen ($P = 0.049$), tPA antigen ($P = 0.003$), sEPCR ($P = 0.009$) and fibrinogen level ($P < 0.0006$) in untreated hypertensive patients was found. The values of laboratory variables in a group of healthy subjects and in all the hypertensive patients are shown in Table 2.

A significant decrease of the BP (SBP and DBP) ($P = 0.000$, respectively) was achieved. All the values of BP and haemostatic parameters were investigated and significance of their changes are shown in Table 3. In comparison with the values before therapy, a significant decrease in plasma vWF ($P = 0.028$), sPsel ($P = 0.029$), sGpV ($P = 0.032$), PAI-1 antigen ($P = 0.043$) and t-PA antigen ($P = 0.040$) after 1 month of perindopril therapy was found. On the other hand, a significant decrease of plasma sEPCR ($P = 0.045$) and fibrinogen level ($P = 0.015$) after 1 month of telmisartan therapy was observed. Changes in all laboratory markers of prothrombotic state investigated after perindopril or telmisartan therapy are shown in Figure 1. However, we failed to find any significant changes of plasma TM, tTG and PF4 due to any therapy investigated. No significant changes of serum chromogranin level due to perindopril or telmisartan therapy were found.

Discussion

Modern antihypertensive therapy has focused not only on BP control but also on the favourable modification of known prognostic indices, such as endothelial and platelet dysfunction or coagulation and fibrinolytic abnormalities. In our study, besides a normalization of high BP, a significant decrease of plasma vWF antigen, sPsel, sGpV, PAI-1 antigen and tPA antigen level after 1 month of perindopril therapy as well as a significant decrease of sEPCR and fibrinogen level after 1 month of telmisartan therapy was observed. This finding can document a beneficial effect of both treatment strategies on prothrombotic state in our hypertensive patients.

In agreement with some previous results, we found the increase of vWF in patients with mild-to-moderate hypertension. It is considered as a predictor of cardiovascular disease. A significant decrease of vWF ($P = 0.028$) after perindopril therapy can indicate the improvement of endothelial function in patients with hypertension. In contrary, we failed to find any significant changes of vWF in hypertensive patients treated by telmisartan. It is reported that only antihypertensive treatment by perindopril but not telmisartan was able to improve conduit artery endothelium-dependent vasodilatation. This beneficial effect of perindopril may be related to bradykinin-dependent mechanisms. Nowadays, it is not clear whether ARBs have beneficial effects comparable to those of ACE inhibitors. Data from EUROPA study and PERTINENT sub-study suggest that long-term (1 year) ACE inhibition with perindopril 8 mg per day exerts a direct positive effect on the vascular endothelium. The results of our present study demonstrate that also a short-term that is 1-month administration of perindopril in a half of the dose used in EUROPA study has a benefit on endothelial function, even in patients with lower risk (without manifest coronary artery disease).

Similarly, the results of our study demonstrate the borderline increase of plasma TM ($P = 0.05$), another marker of endothelial cell injury, in hypertensive patients. The TM is released into the plasma only by true endothelial cell damage during development of vascular complications and probably a certain degree of endothelial injury is necessary for its plasma increase. In this study, only early stage hypertensive patients were included for investigations. This may be the explanation that no changes in plasma TM were found during 1-month treatment by perindopril and telmisartan in our study.

In our hypertensive patients, we found the increased sPsel ($P = 0.015$) and sGpV ($P = 0.015$), which are both considered to be plasma markers of in vivo platelet activation. The increased sPsel is a predictor of adverse cardiovascular events. Soluble GpV is significantly elevated in patients with atherothrombotic diseases. The levels of sPsel and sGpV were significantly decreased only after perindopril ($P = 0.029$ and 0.032, respectively), but not by telmisartan therapy. It is reported that antihypertensive therapy with an ACE inhibitor can result in a reduction in plasma levels of sPsel.

**Figure 1** Changes of haemostatic variables investigated (markers of prothrombotic state) in hypertensive patients due to 1-month therapy by perindopril or telmisartan. A decrease of von Willebrand factor (vWF), soluble P-selectin (sPsel), soluble glycoprotein V (sGpV), plasminogen activator inhibitor type 1(PAI-1) antigen, tissue type plasminogen activator (tPA) antigen ($P < 0.05$, respectively) by perindopril therapy, and a decrease of soluble endothelial protein C receptor (sEPCR) and fibrinogen (Fbg) ($P < 0.05$, respectively) after telmisartan therapy is shown. 

* $P < 0.05$—ine comparison with the baseline values (compared by the Student’s paired t-test). TM, thrombomodulin; tTG, β-thromboglobulin; PF4, platelet factor 4.
the other hand, no significant changes of βTG and PF4—other platelet-specific proteins released from the α-granules, were found in our hypertensive patients. The improvement of platelet function in hypertensive patients treated by perindopril is in accordance with our previous findings of decreased platelet aggregation.21 This is related to findings that perindoprilat (but not candesartan) enhances inhibition of platelet activation by augmenting endothelial anti-platelet properties.31 It seems, that exogenous angiotensin II does not modify platelet activation.32 Platelet activation may be suppressed, in the presence of intact endothelial cells, through increased prostacyclin and nitric oxide release, induced by elevated bradykinin levels due to ACE inhibition. This last mechanism is absent in patients treated by ARB. A subsequent reduction of catecholamine release by antihypertensive therapy could be a further factor for a favourable effect on platelet function. However, this cannot be supported by our results, since no changes in chromogranin level between any groups were found.

In untreated hypertensive patients of our study, the increased levels of PAI-1 antigen (P = 0.049) and t-PA antigen (P = 0.003) were found. Increased PAI-1 and tPA antigen levels can be independently associated with hypertension.33 The mechanism of impaired fibrinolysis in hypertension may be related to endothelial dysfunction. The PAI-1 is produced by endothelial cells34 and by adipose tissue.35 Angiotensin II (via AT₁ receptor) but also angiotensin IV can stimulate the release of PAI-1 from endothelial cells.5,6,36 It is anticipated that ARBs may impair the fibrinolytic system due to increased angiotensin IV.5 The ACE inhibitors are thought to favourably affect the fibrinolytic balance by decreasing angiotensin II-mediated PAI-1 release and/or by increasing bradykinin-induced tPA release from endothelial cells.18 On the basis of our results, pro-fibrinolytic effect of perindopril (but not of telmisartan) was documented. After 1 month of perindopril treatment, a significant decrease of PAI-1 antigen (P = 0.043) as well as tPA antigen (P = 0.040) was observed. Perindopril increases the ability of bradykinin to stimulate the release of tPA in the human coronary circulation, which is not seen with losartan.18 To date, controversial results have been reported about the effect of various ARBs but also of ACE inhibitors on fibrinolysis.8,36–39 The different effects of each drug can be associated with the changes in angiotensin metabolites. Anyway, telmisartan did not attenuate fibrinolysis. This is related to similar in vitro findings with telmisartan on PAI-1 release in cultured mesangial cells, possibly owing to its lipophilic and antioxidant properties.40 Our results are in accordance with findings of other authors, showing that levels of PAI-1 antigen are reduced after antihypertensive treatment with perindopril.41,42 A significant increase of sEPCR in hypertensive patients (P = 0.009) and a decrease of sEPCR due to telmisartan therapy (P = 0.045) in our study is a new finding. The present study offers one of the first evidence that hypertension can contribute to the increased sEPCR levels. The EPCR is expressed mainly on the endothelium of large vessels.43 The release of sEPCR is inducible by inflammation and/or thrombin generation.44 In our patients, who were free of evident inflammatory disease, plasma levels of sEPCR can be a potential marker for the prothrombotic state. According to our results, it is probably possible to improve this prothrombotic state by telmisartan. The mechanism of this effect is not clear; but because of the endothelial cell receptor expression, a speculation about the improvement of functional properties of large arteries can arise as explanation.

Hypertensive patients are known to have abnormalities of rheological function. Plasma fibrinogen is an important determinant of blood viscosity and its high plasma levels are observed in hypertensive patients,9 particularly in nondippers.13 Previous studies examining the influence of therapeutic intervention on fibrinogen plasma levels have shown conflicting results: for example, no changes after losartan,10–12 telmisartan,14–16 enalapril,17–19 perindopril14 but decrease of fibrinogen with irbesartan.20 It has been suggested that changes in fibrinogen level may result from haemodilution caused by vasodilating agents or by a decrease in red cell rigidity.46

In conclusion, the data presented here may offer an additional explanation for the efficacy of the RAS targeting agents in the prevention of cardiovascular events in patients with atherosclerotic vascular disease. Our study demonstrates a beneficial effect of both perindopril and telmisartan on prothrombotic state, in addition to their efficacy to normalize elevated BP. The potentially antithrombotic effect of the RAS targeting agents (vasculoprotective, anti-platelet and profibrinolytic effects of perindopril and anticoagulant/rheological effects of telmisartan) may in turn support the preservation of cardiovascular function. Whether all ACE inhibitors and ARBs possess similar antithrombotic properties remains an open question.
Acknowledgements
We thank Mrs V Tokarova, Mrs H Mackovychova and Mrs V Volanska for their technical assistance. This research was supported by grants from the Slovak Ministry of Education (VEGA grant nos. 1/2290/05 and 1/4301/07).

References
9 Remko M. Acidity, lipophilicity, solubility, absorption, and polar surface area of some ACE inhibitors. Chem Pap 2007; 61: 133–141.


