Endothelium-dependent vasodilatation in forearm is impaired in stroke patients

A. STENBORG1, A. TERENT1 & L. LIND1,2
From the Department of 1Medical Sciences, Uppsala University, Uppsala; and 2AstraZeneca R&D, Mölndal; Sweden


Objectives. To investigate endothelium-dependent vasodilatation (EDV) in stroke patients.

Design. Cross-sectional.

Setting. University referral hospital.

Subjects. We studied 23 stroke patients (65–75 years old) who did not have atrial fibrillation or Warfarin treatment. Forty-six age- and sex-matched healthy controls and another 32 controls matched also for hypertension and medication were used for comparisons.

Methods. Endothelium-dependent vasodilatation was measured with venous occlusive plethysmography of forearm blood flow during intra-brachial infusion of acetylcholine. Endothelium-independent vasodilatation (EIDV) was evaluated by infusion of sodium nitroprusside.

Results. Stroke patients showed significantly lower EDV (P < 0.001) compared with healthy controls when measured with acetylcholine-stimulated forearm blood flow. The difference between these groups remained significant also after correction for blood pressure, body mass index, blood glucose and cholesterol. There was also significant difference in EDV between hypertension-matched controls and healthy controls. However, EIDV was significantly reduced in stroke patients (P < 0.01), but not in the hypertensive group, when compared with healthy controls.

Conclusion. An impaired EDV was seen in both stroke patients and hypertension-matched controls, while an impaired EIDV was seen in the stroke patients only, suggesting a more severe vasodilatory dysfunction in stroke patients than could be explained by a high blood pressure only.

Keywords: endothelium, hypertension, stroke.

Introduction

Stroke is mainly a disease of the arterial circulation of the brain. Hypertension is the most important risk factor, and antihypertensive medication is effective for primary and secondary prevention [1]. Considering the large population of hypertensive patients, it is important to be aware of associated risk factors, which can identify the patients who have the highest risk, in order to be effective in stroke prevention. Impairment in endothelium dependent vasodilatation, endothelium-dependent vasodilatation (EDV) could be such a risk factor. There are both experimental and clinical studies, which indicate that impairment of EDV might be of importance in the pathogenesis of stroke. The spontaneously stroke-prone hypertensive rat shows impaired EDV compared with the spontaneously hypertensive rat [2]. Impaired EDV was associated with a four-fold increased risk of stroke or transient ischaemic attack (TIA) during follow-up in a group of patients who had been evaluated for coronary artery disease by coronary angiogram, but who did not show significant stenosis in the coronary arteries [3].

Endothelium-dependent vasodilatation is a basic physiologic feature for arteries, and dysfunction has been shown to proceed overt atherosclerosis development [4] and future cardiovascular events [5–7].
There are several ways of investigating EDV. For the evaluation of coronary endothelial function, the standard method is infusion of acetylcholine into coronary arteries during coronary angiography. This method is, however, restricted to patients undergoing coronary angiography for clinical reasons. Less invasive alternatives are evaluation of forearm blood flow (FBF) using venous occlusion plethysmography [7–9] and ultrasonographic examination of the brachial artery during hyperaemia to measure flow-mediated vasodilatation [10, 11]. Applanation tonometry, used for pulse wave analyses, is a more recently developed method to evaluate EDV [12, 13].

Although EDV can be evaluated in retinal vessels, which are morphologically and functionally related to cerebral blood vessels, no established method exists to evaluate EDV in the cerebral circulation in humans. One method, which has been used in stroke patients, is systemic administration of L-arginine and evaluation of cerebral flow by transcranial Doppler. Zvan et al. [14] demonstrated impaired flow velocity in the affected hemisphere of patients with recent stroke. Opposite results were, however, achieved in another study showing enhanced cerebral L-arginine-reactivity in patients with a history of stroke or TIA [15].

The current study was undertaken to evaluate if impaired EDV in the peripheral circulation is a characteristic feature of patients with cerebrovascular disease. As hypertension is present in the majority of stroke patients and since an impaired EDV is seen in uncomplicated hypertension [7, 9, 16], the stroke patient group was compared not only with a healthy control group, but also with a control group matched for hypertension.

Methods

Study sample

Stroke patients aged 65–75 years, living in the Uppsala area, Sweden, were identified from the Swedish National Stroke Register (http://www.riks-stroke.org). They were invited to participate in the study unless they had atrial fibrillation, CT-findings of cerebral infarctions in different vascular territories indicating cardioembolic stroke, or were treated with Warfarin. Of the 23 stroke patients included, four had had haemorrhagic stroke, and the remainder had suffered a cerebral infarct. Time from stroke to examination varied between 3 and 25 months (median 11 months).

Controls were selected from a cohort of 70-year-old men and women from Uppsala who were invited to participate in a cardiovascular health investigation: The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study http://www.medsci.uu.se/ pivus/pivus.htm) [17].

The healthy control group \((n = 46)\) included subjects without history of cardiovascular disease. They had no regular medication and were non-smokers. The hypertension-matched control group \((n = 32)\) consisted of subjects free from the history of stroke, but was frequency-matched regarding hypertension and antihypertensive treatment, history of myocardial infarction and smoking when compared with the stroke patient group. The Human Ethics Committee of Uppsala University approved the protocol and the participants gave informed consent.

Experimental procedures

Participants were investigated in the morning or at midday after >4 h of fasting. All medication was withheld the day of examination. An arterial catheter was inserted in the brachial artery for regional infusions. Arterial blood was taken for analyses of fasting blood glucose and lipids.

The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography before and at the end of the infusion of different dosages of the two vasodilators. A mercury in-silastic strain gauge was placed at the upper third of the forearm, which rested comfortably slightly above the level of the heart. The strain gauge was connected to a calibrated plethysmograph. Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mmHg by a rapid cuff inflator. Evaluations of FBF were made by calculations of the mean of at least five consecutive recordings.

After evaluation of resting FBF, local intra-arterial drug-infusions were given during 5 min for each dose with a 20-min washout period between the drugs. The infused dosages were 25 and 50 \(\mu\)g min\(^{-1}\) for acetylcholine (Clin-Alpha, Laefulingen,
Switzerland) to evaluate endothelium-dependent vasodilatation (EDV) and 5 and 10 \( \mu g \) min\(^{-1}\) for sodium nitroprusside (SNP; Nitropress, Maidenhead, Abbot, UK) to evaluate endothelium-independent vasodilatation (EIDV). The dosages of these drugs have been chosen to result in augmentation of FBF on the steep part of the dose–response curve. The drugs were given in a random order at a maximal rate of 1 mL min\(^{-1}\).

As the primary measure of endothelium-dependent vasodilation, EDV was defined as FBF during infusion of 50 \( \mu g \) min\(^{-1}\) of acetylcholine minus resting FBF divided by resting FBF and EIDV was defined as FBF during infusion of 10 \( \mu g \) min\(^{-1}\) of SNP minus resting FBF divided by resting FBF. As a secondary analysis, FBF during the vasodilations are also given as absolute values.

**Pulse wave analysis**

In the assessment of the pulse wave, a micromanometer tipped probe (Sphygmocor, Pulse Wave Medical Ltd, Melbourne, Australia) was applied to the surface of the skin overlying the radial artery and the peripheral radial pulse wave was continuously recorded. For accurate recordings, the micromanometer must be applied with a light pressure to flatten the vessel walls so that the transmural forces within the vessel are perpendicular to the arterial surface. The mean values of around 10 pulse waves were used for analyses. The maximal systolic peak and the reflected waves (Fig. 1) were identified by the calculations of first and second derivative of the different parts of the pulse curve by the commercial software supplied by the manufacturer. In the present study, the relative height of the first diastolic reflected wave, the reflectance index (RI), is used as an index of EDV, as previously validated [12]. After a baseline recording, 0.25 mg terbutaline was subcutaneously administered and a re-evaluation of the pulse wave was performed after 15 and 20 min. It has previously been found that the maximal reduction in the first reflected diastolic wave occurs after 15 min in young healthy subjects [12], but we also recorded this phenomenon after 20 min in our sample of elderly subjects.

Endothelium-dependent vasodilatation evaluated by this technique was defined as the lowest RI-value obtained after 15–20 min following terbutaline minus the baseline value in relation to the baseline value. Reflectance index has recently been found to be related to coronary risk [17].

**Statistics**

Differences between groups were evaluated by factorial ANOVA, with Bonferroni post hoc analyses. When the relationships between vasodilatory variables and the three groups were adjusted for the potential confounding variables fasting blood glucose, serum cholesterol, smoking, body mass index (BMI) and gender and blood pressure, ANCOVA was used. Spearman’s correlation coefficient was used to relate time from the stroke event to the examination to the vasodilatory indices. Two-tailed \( P \)-values are given with \( P < 0.05 \) regarded as significant.

**Results**

Demographics and risk factors are given in Table 1. The groups were matched for age and gender, but significant differences between the three groups
Endothelium-dependent vasodilatation

As could be seen in Fig. 1, EDV evaluated by the invasive forearm technique differed between the groups \((P < 0.001)\) by ANOVA analysis. In post hoc analysis, stroke patients showed a reduced EDV when compared with healthy controls \((P < 0.001)\), but not in comparison with hypertension-matched controls \((P = 0.27)\). Also, the hypertension-matched control group showed a reduced EDV when compared with the control group \((P < 0.01)\), see Fig. 1). In multiple ANCOVA analysis with EDV as the dependent variable and group together with the potential confounding variables fasting blood glucose, serum cholesterol, smoking, BMI and gender and blood pressure as independent variables, the difference between stroke patients and healthy controls was still significant (mean \(±\ SEM: 325 ± 38\) vs. \(691 ± 60\%), \(P < 0.001\)). Also, the difference between hypertension-matched group \((429 ± 41\%)\) and healthy controls persisted after this adjustment \((P = 0.004)\). Adding aspirin use to the model did not substantially change the results described above and all described differences were still highly significant.

Also when endothelium-dependent vasodilation was evaluated with the absolute number of FBF during infusion with acetylcholine in secondary analysis, both the stroke group and the matched-hypertensive group were significantly impaired in acetylcholine-mediated vasodilation compared with the control group (see Fig. 2 for details).

### Table 1: Demographic and risk factors in stroke patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Stroke patients</th>
<th>Healthy controls</th>
<th>Hypertension-matched controls</th>
<th>ANOVA P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>23</td>
<td>46</td>
<td>32</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.5</td>
<td>70.0</td>
<td>70.0</td>
<td>–</td>
</tr>
<tr>
<td>Females (%)</td>
<td>43</td>
<td>41</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>25.5 ± 3.7</td>
<td>24.3 ± 2.6</td>
<td>28.3 ± 4.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol L(^{-1}))</td>
<td>5.4 ± 1.6</td>
<td>4.9 ± 0.5</td>
<td>6.2 ± 2.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Serum cholesterol (mmol L(^{-1}))</td>
<td>5.2 ± 0.9</td>
<td>5.2 ± 0.7</td>
<td>5.4 ± 1.0</td>
<td>0.52</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>154 ± 24</td>
<td>126 ± 12</td>
<td>158 ± 25</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84 ± 8</td>
<td>71 ± 8</td>
<td>83 ± 13</td>
<td>0.0001</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Antihypertensive medication (%)</td>
<td>80</td>
<td>0</td>
<td>78</td>
<td>–</td>
</tr>
<tr>
<td>History of myocardial infarction (%)</td>
<td>8.7</td>
<td>0</td>
<td>6</td>
<td>–</td>
</tr>
</tbody>
</table>
For EIDV, there was a difference between the three groups \((P = 0.02)\) by ANOVA analysis. In post hoc analysis, there was a significant difference between stroke patients and healthy controls \((P = 0.006)\), but not between stroke patients and hypertension-matched controls \((P = 0.15)\). The hypertension-matched control was not different in EIDV compared with the healthy control group \((P = 0.16)\).

In multiple ANCOVA analysis with EIDV, as the dependent variable and group together with the potential confounding variables fasting blood glucose, serum cholesterol, smoking, BMI and gender and blood pressure as independent variables, the difference between stroke patients and healthy controls was still significant \((mean ± SEM: 270 ± 24 vs. 447 ± 39\%, P < 0.01)\). Adding aspirin use to the model did not substantially change the results described above and the described difference was still significant.

When endothelium-independent vasodilatation was evaluated with the absolute number of FBF during infusion with SNP in secondary analysis, only the stroke group was impaired in SNP-mediated vasodilation compared with the control group, although not statistically significant \((P = 0.07, see Fig. 2 for details)\).

**Pulse wave analysis**

Also for the pulse wave analyses, there was a difference between groups regarding the change in RI following terbutaline \((P = 0.015)\) by ANOVA analysis \((Fig. 3)\), with a significant difference between stroke patients and healthy controls \((P = 0.007)\) in post hoc analysis, but not between stroke patients and hypertension-matched controls \((P = 0.33)\) or between hypertension-matched controls and healthy controls \((P = 0.051)\).

In multiple ANCOVA analysis with the change in RI as the dependent variable and group together with the potential confounding variables fasting blood glucose, serum cholesterol, smoking, BMI and gender and blood pressure as independent variables, the difference between stroke patients and healthy controls was no longer significant \((mean ± SEM: −27 ± 4.5 vs. −35 ± 2.3\%, P = 0.09)\).

A significant relation between the time from the stroke event and the examination was found regarding EDV \((r = 0.47, P = 0.046)\), but not regarding EIDV or the change in RI \((P-value: 0.82 and 0.48 respectively)\).

**Discussion**

In this study, stroke patients showed impaired acetylcholine-mediated vasodilatation compared with age- and sex-matched healthy controls. This difference in EDV was still significant after adjustments for fasting blood glucose, serum cholesterol, BMI and blood pressure. An impaired EDV was also seen in the hypertension-matched control group when compared with healthy controls.

However, EIDV was significantly reduced in stroke patients, but not in the hypertensive group, when compared with healthy controls, suggesting a more severe vasodilatory dysfunction in stroke patients than could be explained by a high blood pressure only.

The main explanation for the impaired EDV in the stroke group is most likely the high prevalence of hypertension. Hypertension induces a profound impairment in EDV[7, 9, 16], which makes it difficult to detect the effects of additional risk factors or diseases, such as stroke.

Treatment with low-dose aspirin has been shown to improve EDV in hypertensive patients [18]. It has been proposed as one of the explanations for the cardiovascular benefit of low-dose aspirin. Acute infusion of acetylsalicylate–lysine has also been reported to improve EDV in humans [19]. Therefore, it is possible that a high prevalence of aspirin treatment in the stroke group (76% vs. 34% in the hypertension-matched control group), might improve EDV in the stroke group, and in that way...
contribute to the lack of significant difference between the stroke group and the hypertension-matched control group. However, adjustment for aspirin usage did not influence the results.

In addition to the impaired EDV seen both in the stroke patients and the hypertensive-matched control group, EIDV was impaired in the stroke group only. Thus, the vascular impairment seen in stroke patients is not only endothelium-dependent, but also includes the vasodilatory process induced by exogenous given nitric oxide. This indicates that the stroke patients, in opposition to the hypertensive group, have a dysregulation in the vasodilatory steps following the exposure of the vessel to nitric oxide. Such a dysfunction could either been due to an impaired vascular smooth muscle function or to structural alterations in the vessel wall not permitting adequate vasodilation. Further studies are needed to clarify these mechanisms in stroke patients.

In the stroke group, a relation between the time from the stroke event and the examination was found regarding EDV, but not regarding EIDV and the pulse wave based method. Thus, it seems as early endothelial dysfunction following the stroke event improves over time. This also highlight the need to take this period into account in future studies examining EDV in stroke patients.

Although the main analysis showed that endothelium-dependent vasodilation was impaired in stroke patients compared to healthy controls using both EDV and the change in RI, the results obtained by the pulse wave based method was less consistent and disappeared after adjustment for common risk factors. Pulse wave reflections are dependent on both stiffness in the aorta and large vessels, as well on peripheral resistance in the reflected vascular beds. Thus, the change in RI is possibly not as clear-cut technique to evaluate endothelium-dependent vasodilation as EDV, although the results are related [17]. In a recent study from the PIVUS cohort, EDV was found to be more closely related to the Framingham risk score than the change in RI [17], exemplifying that EDV is the preferred technique.

The present study evaluated endothelial function in the peripheral circulation. How these results relate to endothelial function in the cerebral circulation is not known. Only a few studies have been devoted to estimations of endothelial function in the cerebral circulation in stroke patients [14, 15] and further studies are needed to compare peripheral and cerebral vascular function.

A limitation of the present study is that motor function was not evaluated in detail in the arm in which plethysmography was performed, although no arm with severe paresis was investigated. Furthermore, similar results were obtained with local and systemic investigation of EDV (occlusion plethysmography of forearm blood flow and pulse wave analysis respectively), suggesting that any minor deterioration in local motor function would not be of major importance for the assessment of EDV.

In conclusion, an impaired EDV was seen in both stroke patients and hypertensive-matched controls, while an impaired EIDV was seen in the stroke patients only, suggesting a more severe vasodilatory dysfunction in stroke patients than could be explained by a high blood pressure only.

Conflict of interest statement

No conflict of interest was declared.

References


Correspondence: Anna Stenborg, Medicinarkivet, Akademiska sjukhuset, S-751 85 Uppsala, Sweden (fax: +4618 6114365; e-mail: anna.stenborg@medsci.uu.se).