Effects of Cilazapril on the Cerebral Circulation in Spontaneously Hypertensive Rats

Jean-Paul Clozel, Herbert Kuhn, and Fridolin Hefti

Chronic hypertension is associated with a lower cerebral vascular reserve due to thickening of the media of cerebral vessels. The goal of the present study was to determine if long-term inhibition of angiotensin converting enzyme with cilazapril, a new long-acting angiotensin converting enzyme inhibitor, could improve cerebral vascular reserve. For this purpose, two groups of 12 spontaneously hypertensive rats were compared. One group was treated with 10 mg/kg/day cilazapril from 14 weeks to 33 weeks of age and was compared with a group treated with placebo. A third group of 12 Wistar-Kyoto rats treated with placebo was used as reference. At the end of the treatment period, cerebral vascular reserve was evaluated by measuring cerebral blood flow (radioactive microspheres) at rest and during maximal vasodilatation induced by seizures provoked by bicuculline. Then, the rats were perfusion-fixed, and morphometry of the cerebral vasculature was performed. Cerebral vascular reserve was severely impaired in the spontaneously hypertensive rats since their maximal cerebral blood flow was decreased by 52% compared with the Wistar-Kyoto rats. Cilazapril normalized cerebral blood flow reserve. This normalization was associated with a decreased thickness of the medial layer in the carotid artery, the middle cerebral artery, and in the pial arteries larger than 100 µm. Further studies are required to determine whether this decreased medial thickness is due to the normalization of blood pressure induced by cilazapril or to the reduction of trophic factors such as angiotensin II. (Hypertension 1989;14:645-651)
inhibitor\textsuperscript{18-22} that is very effective in prevention of the decrease of coronary vascular reserve in SHR.\textsuperscript{23} Therefore, the goals of the present study were to determine whether long-term treatment with cilazapril could normalize the cerebral blood flow reserve in SHR and which morphological changes were induced by cilazapril along the cerebral vascular bed.

Materials and Methods

Animals

Three groups of rats were compared. One group of 12 normotensive male Wistar-Kyoto (WKY) rats was used as reference. Another group of 12 male SHR from the Okamoto strain (SHR/SP/A3N) was treated with placebo. A third group of 12 male SHR received 10 mg/kg/day of cilazapril as food admixture. The three groups of rats were maintained under identical conditions of temperature (20–22°C), humidity (50–60%), and photoperiod, had free access to normal rat chow and water, and were treated from 14 weeks to 33 weeks of age. Systolic arterial pressure was measured at the beginning, after 1 month, and at the end of the treatment period with an indirect tail-cuff method.

Measurement of Cerebral Blood Flow Reserve

Cerebral blood flow reserve was measured at the end of the treatment period. The rats were anesthetized with 30 mg/kg pentobarbital, and then the thorax was opened and a polyethylene catheter (PE 10) was implanted into the left atrium for injection of radioactive microspheres. Another catheter was implanted into the abdominal aorta through the femoral arterial catheter with a pressure transducer (model 4.422, Bell & Howell, Basingstoke, England), and heart rate, measured from the arterial pressure trace with a tachymeter, were recorded by a Watanabe recorder (Watanabe, Tokyo, Japan). Each microsphere injection was made when arterial pressure was stable. Moreover, for each microsphere injection it was checked that arterial pressure was similar before and after the microsphere injection. However, we cannot exclude that transient changes of arterial pressure occurred during the microsphere measurement.

At the end of the study, the rats were heparinized and killed with an overdose of pentobarbital. The brain was perfusion-fixed in preparation for morphometry and removed and dissected to separate the cerebellum from the rest of the brain.

Radioactivity of the brain was measured in a gamma camera equipped with a germanium crystal (Enertec, Strasbourg, France).

Cerebral blood flow was calculated by the following equation:

\[ \text{Cerebral blood flow} = \frac{Q_r \times NS}{NR} \]

where \( Q_r \) is the reference sample withdrawal rate, \( NS \) is the number of microspheres in the tissue sample, and \( NR \) is the number of microspheres in the reference sample.

Cerebral vascular resistance was calculated as mean arterial pressure (measured just before the microsphere injection) divided by cerebral blood flow.

To measure cerebral blood flow reserve, cerebral blood flow was measured before and after induction of seizures with 1 μg/kg bicuculline. Induction of seizures has been shown to induce maximal cerebral blood flow increase in rats.\textsuperscript{25,26} In addition, the rats were bled before injection of bicuculline, until a mean arterial pressure of around 40 mm Hg was obtained, to prevent the extreme increase of arterial blood pressure during the seizures.

Morphometry of the Microvasculature

Morphological measurements were made on perfusion-fixed vessels and brain. After each rat was killed, a cannula was implanted into the left ventricle; then the rat was perfused at a pressure of 90 mm Hg, first with a Krebs-Henseleit solution containing 10^{-5} M adenosine, then with phosphate-buffered glutaraldehyde (2.5%). In vivo injection of a similar dose of adenosine has been previously shown to induce maximal cerebral vasodilation in vivo.\textsuperscript{27} The carotid artery and the brain were removed and, after dehydration with ethanol and xylol and embedding in paraffin, 1.5 μm cross sections of the brain were cut, mounted on glass slides, and stained with hematoxylin-eosin.

The slides were examined with a video camera (model KY 1900E, JVC, Tokyo, Japan) mounted on a standard microscope (Universal Zeiss, Oberkochen, FRG). Morphological analysis was performed at a magnification of ×1,000 for capillaries and small arteries and ×500 for larger arteries. Drawings of the limits of the vessels were made on the screen of a video monitor (model PVM 1371 QM, Sony, Tokyo, Japan).
**Study Design and Statistical Analysis**

To evaluate the effects of cilazapril, the SHR treated with cilazapril were compared with the SHR treated with placebo with unpaired *t* test. The group of normotensive WKY rats was used as a reference group. However, it should be noted that the differences between SHR and WKY rats cannot be attributed solely to hypertension because genetic differences could also play a role.

**Results**

**Hemodynamic Variables**

The systolic arterial pressure results of the three groups of rats during the study is shown in Figure 1. In the WKY rats, systolic arterial pressure was stable around 140 mm Hg during the study. In the SHR treated with placebo, systolic arterial pressure remained elevated around 210 mm Hg. Cilazapril normalized systolic arterial pressure in the SHR. Systolic, diastolic, and mean arterial pressure measured directly on the day of the cerebral blood flow determination were decreased by cilazapril (Table 1). Despite this decrease of arterial pressure, there was no reflex tachycardia (Table 1). During the seizures, mean arterial pressure was higher in the SHR treated with placebo than in the SHR treated with cilazapril, although the preseizure value was made identical by bleeding (Table 1).

**Body and Brain Weights**

Body and brain weights (expressed in absolute value or as the ratio brain weight/body weight) are shown in Table 1. The body weight was similar in the three experimental groups. In contrast, the brain weight was slightly smaller in the SHR versus the WKY rats. Cilazapril did not normalize the brain weight.

**Cerebral Blood Flow Reserve**

Cerebral blood flow and vascular resistances values are listed in Table 2. Before seizures, the baseline cerebral blood flow was similar in the three experimental groups. In contrast, the brain weight was slightly smaller in the SHR versus the WKY rats. Cilazapril did not normalize the body weight.

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**Figure 1.** Line graph showing effect of cilazapril on systolic arterial pressure (SAP) in three experimental groups of rats. ***p<0.001 versus spontaneously hypertensive rats (SHR) treated with placebo. WKY, Wistar-Kyoto rats.

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**Table 1.** Body Weight, Brain Weight, and Hemodynamic Variables in Three Experimental Groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body wt (g)</th>
<th>Brain wt (g)</th>
<th>Brain wt/body wt</th>
<th>Arterial pressure (mm Hg)</th>
<th>Systolic</th>
<th>Diastolic</th>
<th>Mean</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR B 12</td>
<td>403±12</td>
<td>1.77±0.02*</td>
<td>4.41±0.12†</td>
<td>173±8*</td>
<td>131±6*</td>
<td>150±7*</td>
<td>392±9*</td>
<td>392±9*</td>
</tr>
<tr>
<td>SHR S 12</td>
<td>395±9</td>
<td>1.80±0.03</td>
<td>4.59±0.11</td>
<td>143±9</td>
<td>110±7</td>
<td>132±6</td>
<td>154±12</td>
<td>154±12</td>
</tr>
<tr>
<td>SHR + cilazapril</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>395±9</td>
<td>1.80±0.03</td>
<td>4.59±0.11</td>
<td>143±9</td>
<td>110±7</td>
<td>132±6</td>
<td>154±12</td>
<td>154±12</td>
</tr>
<tr>
<td>WKY B 12</td>
<td>428±9</td>
<td>2.06±0.05</td>
<td>4.85±0.12</td>
<td>102±5</td>
<td>74±5</td>
<td>90±5</td>
<td>327±12</td>
<td>327±12</td>
</tr>
<tr>
<td>S 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>138±6</td>
<td>99±6</td>
<td>121±5</td>
<td>200±25</td>
</tr>
</tbody>
</table>

Arterial pressure and heart rate were measured during anesthesia with a direct method at baseline (B) and during seizures (S). Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; NS, not significant.

†, *p<0.05, p<0.001 versus WKY rats.

§, #p<0.05, p<0.001 versus placebo-treated SHR.

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**Methods**

Japan and then digitized (DIASYS-system, dataLab, Thoeringen, Switzerland).

The carotid artery, the middle cerebral artery, and cerebral pial arteries were studied. Because of the low number of cerebral pial arteries, three slices of the brain were studied for each rat. The wall cross-sectional area and the total area of the vessels (excluding the adventitia) were measured. Because the ranking order of the pial arteries was not determined, to normalize for the size of these arteries the diameter of each pial artery was calculated by measurement of the length of the internal elastic lamina. Because the carotid and the middle cerebral arteries were always studied at the same location, three types of variables could be evaluated: the wall cross-sectional area, the mean thickness of the vessel wall, and the wall/diameter ratio. In contrast, the pial arteries were normalized for their size and classified in two groups (diameter less than 100 μm and between 100 μm and 300 μm). Only the wall/diameter ratio was used for the arteries to take into account the different sizes of arteries. For each size group of arteries, the mean of the wall/diameter ratio was averaged for each rat and used for the statistical analysis.
TABLE 2. Cerebral Blood Flow and Cerebral Vascular Resistances in Three Experimental Groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>n</th>
<th>Cerebral blood flow (ml/min/g)</th>
<th>Cerebral vascular resistance (mm Hg/ml-min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Left brain</td>
<td>Right brain</td>
</tr>
<tr>
<td>SHR B 12</td>
<td>12</td>
<td>0.28±0.02</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>SHR S 12</td>
<td>12</td>
<td>2.27±0.32†</td>
<td>2.17±0.29†</td>
</tr>
<tr>
<td>SHR + cilazapril B 12</td>
<td>12</td>
<td>0.33±0.02</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>SHR + cilazapril S 12</td>
<td>12</td>
<td>4.43±0.72</td>
<td></td>
</tr>
<tr>
<td>WKY B 12</td>
<td>12</td>
<td>0.32±0.02</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>WKY S 12</td>
<td>12</td>
<td>4.74±0.82</td>
<td>4.92±0.81</td>
</tr>
</tbody>
</table>

Cerebral blood flow and cerebral vascular resistance were measured at baseline (B) and during seizures (S). Values are mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

†,†,*,p<0.05, p<0.01, p<0.001 versus WKY rats.
||,||,§,p<0.05, p<0.01, p<0.001 versus placebo-treated SHR.

during seizures, cerebral blood flow increased dramatically in the three groups of rats, but this increase was much less in SHR than WKY rats. Minimal cerebral vascular resistance was higher in every portion of the brain in the SHR compared with the WKY rats (p<0.001). Cilazapril normalized completely maximal cerebral blood flow and minimal cerebral vascular resistance.

**Morphological Measurements**

The carotid (Figure 2A and B) and the middle cerebral arteries were fixed in relaxation as shown by the regular circular aspect and the internal elastica lamina. The results of the morphological measurements are shown in Table 3. The medial layer of the carotid artery was thicker in the SHR compared with the WKY rat. Thickening of the medial layer was not as marked in the middle cerebral artery. Cilazapril normalized the size of the medial layer of carotid and middle cerebral arteries. The medial layer of large pial arteries (between 100 and 300 µm diameter) was also thicker in SHR than in WKY rats. Cilazapril also reduced the thickness of the medial layer in these large pial arteries. However, thickening of the media was not present in the smaller (less than 100 µm) pial arteries of SHR, and cilazapril had no

**Figure 2.** Light micrograph of semithin cross sections of rat carotid arteries. Panel A: Carotid artery of spontaneously hypertensive rat (SHR) treated with placebo. Panel B: Carotid artery of cilazapril-treated SHR. Arrowheads mark media. Cilazapril decreased significantly thickness of media. Arrow indicates endothelial cell layer. Bars equal 50 µm.
changes such as increases of capillary or artery of the medial layer of the cerebral arteries and WKY rats. Cilazapril completely normalized cerebral arterioles. However, we cannot exclude other resistance was increased in SHR compared with decreased and, in parallel, minimal cerebral vascularization has also been previously observed with cilazapril, captopril, and enalapril. The present study shows that long-term ACE inhibition with cilazapril can normalize the cerebral vascular reserve in SHR, most likely by reversal of the medial thickening that occurs in the cerebral arterial bed.

Cerebral vascular reserve was evaluated by measuring cerebral blood flow and vascular resistance before and during maximal cerebral vasodilatation obtained by induction of seizures. In basal conditions (without seizures), cerebral blood flow depends mainly on vascular tone and tends to stay constant despite changes of perfusion pressure (autoregulation). Cerebral oxygen consumption is an important determinant of cerebral blood flow. Cilazapril is unlikely to have changed cerebral oxygen consumption. This absence of effect on baseline cerebral blood flow has also been previously observed with cilazapril, captopril, and enalapril.

In contrast, during seizures, vasodilation of the cerebral vasculature is maximal. There is no more active vascular tone. Then, the cerebral blood flow depends on the perfusion pressure (aortic pressure) and the cross-sectional cerebral vascular area. Therefore, measurement of the minimal cerebral vascular resistance gives an estimation of the cerebral vascular cross-sectional area.

Our study confirmed previous findings that have shown a decrease of cerebral vascular reserve during hypertension. Maximal cerebral blood flow was decreased and, in parallel, minimal cerebral vascular resistance was increased in SHR compared with WKY rats. Cilazapril completely normalized cerebral vascular reserve.

The improvement of cerebral vascular reserve was most likely due to a decrease of the thickening of the medial layer of the cerebral arteries and arterioles. However, we cannot exclude other changes such as increases of capillary or artery numbers. In the present study, thickening of the medial layer was present in the carotid and middle cerebral artery and in the large (more than 100 μm) arteries but not in the small (less than 100 μm) arteries. Thus, medial thickening seems to decrease downward along the cerebral vascular bed. This is in contrast with what has been described in previous studies in stroke-prone SHR and SHR. However, the morphometry in these studies took into account only the diameter and the thickness of the vessels without measuring the area of the vessel wall. Therefore, it is not excluded that artifacts due to the persistent constriction of the arteries were present. In another study performed in adult SHR rats, only the first- and fourth-order arteries had an increased wall thickness but not the second- and third-order arteries. However, a recent study has shown that hypertension was associated not only with an increased thickness of the media but also with a decrease of the external diameter of the cerebral arterioles. This decrease of the external diameter of the cerebral arterioles plays an important role in explaining the decrease of the cross-sectional area of these arterioles. A “remodeling” of the cerebral arterioles would not have been detected in our study where the cerebral arterioles were not always studied at the same location. This could also explain the absence of statistically significant thickening of the media of small arterioles in SHR.

Moreover, as mentioned in the Methods section, WKY rats have been used only as a reference. The purpose of this study was not to compare directly SHR and WKY rats because genetic differences could explain the differences observed.

Cilazapril normalized the thickness of the cerebral arteries of the SHR. This effect was not due to an artefact of fixation. It could be argued that with cilazapril the cerebral arteries were less constricted during fixation and that the difference of thickening was only because of a difference of constriction. However, to avoid active constriction, the rats

<table>
<thead>
<tr>
<th>Vessel type</th>
<th>Group</th>
<th>n</th>
<th>Cross-sectional area of the media (μm²)</th>
<th>Medial thickness (μm)</th>
<th>Media/arterial diameter thickness</th>
<th>Inner arterial diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery</td>
<td>WKY</td>
<td>11</td>
<td>65,850±2,270</td>
<td>29±1</td>
<td>0.039±0.002</td>
<td>720±18</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>10</td>
<td>92,070±3,430*</td>
<td>36±2†</td>
<td>0.042±0.003</td>
<td>816±28</td>
</tr>
<tr>
<td></td>
<td>SHR+CIL</td>
<td>11</td>
<td>58,480±1,720‡</td>
<td>25±1‡</td>
<td>0.033±0.002‡</td>
<td>746±22</td>
</tr>
<tr>
<td>Middle cerebral artery</td>
<td>WKY</td>
<td>11</td>
<td>9,200±1,150</td>
<td>11±1</td>
<td>0.041±0.002</td>
<td>260±16</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>8</td>
<td>9,590±1,380</td>
<td>13±1</td>
<td>0.053±0.003†</td>
<td>212±16</td>
</tr>
<tr>
<td></td>
<td>SHR+CIL</td>
<td>11</td>
<td>9,640±1,240</td>
<td>11±1</td>
<td>0.044±0.003§</td>
<td>230±22</td>
</tr>
<tr>
<td>Pial arterioles (100–300 μm)</td>
<td>WKY</td>
<td>14</td>
<td>...</td>
<td>...</td>
<td>0.043±0.002</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>15</td>
<td>...</td>
<td>...</td>
<td>0.054±0.003†</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR+CIL</td>
<td>9</td>
<td>...</td>
<td>...</td>
<td>0.044±0.005§</td>
<td>...</td>
</tr>
<tr>
<td>Pial arterioles (&lt;100 μm)</td>
<td>WKY</td>
<td>11</td>
<td>...</td>
<td>...</td>
<td>0.091±0.012</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>11</td>
<td>...</td>
<td>...</td>
<td>0.084±0.008</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>SHR+CIL</td>
<td>10</td>
<td>...</td>
<td>...</td>
<td>0.082±0.010</td>
<td>...</td>
</tr>
</tbody>
</table>

Because the ranking order of the pial arteries was not determined, only the media/arterial diameter thickness ratio was taken into account for these arteries. Values are mean±SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; CIL, cilazapril.
were perfusion-fixed with a medium containing 10^{-5} M adenosine that produced a complete relaxation of the cerebral arteries and arterioles as shown by the absence of convolution of the internal elastica lamina. Moreover, we measured the cross-sectional area of the media of the arteries. Because the surface of the wall does not depend on the constriction state, the beneficial effect of cilazapril must be related to a change of smooth muscle mass.

The present results confirm our previous study, which showed that cilazapril could prevent the decrease of the coronary vascular reserve. Moreover, recently, it has been shown that perindopril, another ACE inhibitor, could decrease the medial thickening and increase the compliance of carotid arteries of SHR. However, in that study as in the present one, it is not possible to differentiate between a regression and a prevention of vascular hypertrophy (however, we did not check this point). Treatment was started when the rats were hypertensive and probably cerebral vascular hypertrophy had already developed. Thus, regression of cerebral vascular hypertrophy must have played a role, but we cannot exclude that prevention of further vascular hypertrophy also occurred.

The design of the present study does not allow us to elucidate the mechanism of action of cilazapril. We do not know whether the thickening of the media was due to hypertrophy (increase in size) or hyperplasia (increase in number) of the smooth muscle cells. To differentiate between these two processes would have required another technique such as the dissector technique. Previously, in SHR it has been shown that hypertrophy occurs in the aorta of SHR, but hyperplasia would explain the medial thickening of mesenteric resistance arteries. In the cerebral arteries, to our knowledge, it is not known whether hypertrophy or hyperplasia occurs.

In the present study, we did not determine whether the effect of cilazapril was due only to a blood pressure decrease or to another effect related to ACE inhibition. Recently, several studies have suggested that in SHR medial hypertrophy precedes the appearance of hypertension and could even be the cause of hypertension. This could explain why the normalization of arterial blood pressure with a pure vasodilator such as hydralazine is not sufficient to reduce medial hypertrophy to a large extent. Moreover, normalization of blood pressure in SHR with the combination of hydralazine, reserpine, and chlorothiazide did not normalize the arterial wall mass of large pial arteries in SHR. The decrease of angiotensin II because of ACE inhibition could explain the effect of cilazapril.

In vitro, angiotensin II induces hypertrophy of smooth muscle cells, and it has been suggested that angiotensin II regulates the growth of the smooth muscle cells of the vessel wall. Moreover, cilazapril has been shown to inhibit ACE in the vessel wall of carotid of rats. However, it is not possible to conclude from our study that the decrease of angiotensin II alone can explain the effects of cilazapril since ACE is not only responsible for the conversion of angiotensin I to angiotensin II but also for the metabolism of bradykinin. Finally, chronic sympathectomy has been shown to reduce the structural component of resistance of large cerebral arteries and ACE inhibitors are known to interfere with the sympathetic system. Thus, we cannot exclude that cilazapril produced its effects via an inhibition of the sympathetic transmission.

The functional consequences of the normalization of cerebral vascular reserve are not known. SHR rats develop larger cerebral infarcts than normotensive rats after ligation of the middle cerebral artery, probably because of the limitation of cerebral vascular reserve. This could be a mechanism involved in the development of strokes. However, it has also been shown in stroke-prone SHR that cerebral vascular hypertrophy could protect cerebral vessels during hypertension. Therefore, further experimental studies are needed to evaluate the possible beneficial effects of the normalization of cerebral vasculature.

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References


**KEY WORDS** • cilazapril • converting enzyme inhibitor • hypertrophy • cerebral blood flow • vascular smooth muscle cells • spontaneously hypertensive rats
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