Effect of cerivastatin on peripheral capillary permeability to albumin and peripheral nerve function in diabetic rats

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Abstract

Objective. – To examine the effect of cerivastatin on capillary permeability to albumin and peripheral nerve function in diabetic rats.

Animals. – Diabetes was induced in male Wistar rats by i.p. injection of streptozotocin (STZ) at the age of 5 days. Forty diabetic rats were randomized in two groups: one treated by cerivastatin (diabetic treated group, DT) and the other untreated (diabetic untreated group, DU). The data were compared to a group of normal rats.

Measurements. – The peripheral capillary filtration of albumin (CFA) was studied on a limb by a non-invasive isotopic method, and nerve electrophysiological measurements were performed. Rats were followed-up until 6 months. In group DU albumin retention (AR) increased by 3 months and lymphatic uptake of interstitial albumin was impaired at 6 months. None of these disorders was observed in group DT. Motor and sensory nerve conduction velocities (MNCV and SNCV) were significantly slower at 6 months in group DU but not in group DT as compared to control rats. The duration of the sensory nerve action potential (SNAP) was significantly longer in group DU than in control rats at 6 months whereas it did not differ in group DT and in control animals.

Conclusions. – This study shows that cerivastatin may prevent the peripheral increase in CFA and lymphatic dysfunction induced by diabetes. These beneficial effects on microcirculation may be involved in the prevention of nerve function deterioration. The underlying mechanisms are likely to be independent of a lipid-lowering effect, but their clarification needs further investigations.

Résumé

Effet de la cérivastatine sur la perméabilité capillaire à l’albumine et la fonction nerveuse périphérique chez des rats diabétiques.

Objectif. – Étudier l’effet de la cérivastatine sur la perméabilité capillaire à l’albumine technétée et la conduction périphérique.

Animaux. – Le diabète a été induit chez des rats Wistar mâles par injection intrapéritonéale de streptozotocine à l’âge de cinq jours. Quarante rats diabétiques ont été randomisés en deux groupes, l’un traité par cérivastatine (groupe DT) et l’autre non traité (groupe DU). Les données ont été comparées à un groupe de rats normaux appariés.

Mesures. – La mesure de la filtration capillaire périphérique d’albumine (CFA) a été effectuée sur une patte par une méthode isotopique non invasive, et des mesures d’électrophysiologie sur le nerf périphérique ont été réalisées. Les rats furent suivis jusqu’à l’âge de six mois. Dans le groupe DU, la rétention d’albumine augmenta dès l’âge de trois mois et la captation lymphatique de l’albumine interstitielle est altérée à six mois. Aucune de ces modifications n’a été observée dans le groupe DT. Les vitesses de conduction nerveuses motrices et sensitives sont significativement plus faibles à l’âge de six mois dans le groupe DU comparé au groupe témoin mais cette diminution n’a pas été retrouvée dans le groupe DT par rapport au groupe témoin. La durée du potentiel d’action sensitif est significativement allongée dans le groupe DU comparé au groupe témoin alors qu’elle est identique dans le groupe DT et le groupe témoin.

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Conclusion. – Cette étude montre que la cérivastatine peut prévenir l’augmentation de la CFA et la dysfonction lymphatique induites par le diabète. Ces effets bénéfiques sur la microcirculation peuvent être impliqués dans la prévention de la détérioration fonctionnelle du système nerveux périphérique. Les mécanismes sous-jacents semblent être indépendants de l’effet hypolipémiant de la cérivastatine, mais ceux-ci méritent d’être clarification par d’autres études.

Keywords: Capillary filtration of albumin; Lymphatic dysfunction; Nerve function; Diabetes; Statins

Mots clés : Filtration capillaire de l’albumine ; Résorption lymphatique ; Nerf périphérique ; Diabète ; Statines

1. Introduction

Diabetes induces macro and microcirculatory complications. Several papers have provided evidence for an endothelial dysfunction in diabetic patients and in animal models of diabetes. Endothelial dysfunction may affect the whole vascular network. We have demonstrated such an endothelial dysfunction in coronary arteries without evidence of atheroma [1] and also in small vessels like skin vessels [2]. An increase in the capillary filtration of albumin (CFA) has been found in diabetic patients and in experimental diabetes by using different methods. In particular, the transcapillary escape rate of albumin has been shown to be increased. By using a non-invasive isotopic method [3,4] we have shown similarly that CFA is increased in diabetic patients [4,5]. This disorder is related to an endothelial leakage of albumin through capillary pores and accounts for endothelial alterations. Metabolic changes consecutive to chronic hyperglycemia are supposed to play an important role in endothelial dysfunction. In particular this role is supported by the preventive effect of inhibitors of the aldose-reductase pathway on CFA increase [6]. Hemodynamic factors are also likely to be involved as suggested by the diminution of CFA under hypertensive treatment [7].

The increase in capillary filtration is supposed to play an important pathophysiological role in diabetic complications. We have previously shown that an increase in CFA is associated significantly with peripheral neuropathy in diabetic patients [8]. This association is consistent with the role of endoneurial swelling in nerve function impairment [9].

Clinical trials of statins have clearly demonstrated an improvement in cardiovascular outcomes. However this improvement is incompletely explained by the baseline or treated LDL-cholesterol level. The beneficial effects of statins may involve nonlipid mechanisms that modify endothelial function, smooth muscle cells, and monocyte-macrophage function, inflammatory responses, and plaque stability. Statin treatment may improve endothelium-mediated vasodilatation in patients with lipid disorders [10,11], at least partly as a result of an antioxidant mechanism [12]. In addition statins may reduce vascular permeability but the effects do not seem homogeneous across the tissues (retina, brain and heart) [13,14] and were tested during only 2 or 5 weeks. Regarding diabetic neuropathy, it has been suggested that rosuvastatin may prevent the impairment in nerve conduction in rats with diabetes induced by streptozotocin (STZ) after 3 months of age by improving neural tissue perfusion [15]. We have recently reported that in rats with early-induced diabetes (STZ administration by 5 days of age), nerve conduction becomes impaired only at 6 months [16] in agreement with the protection of neural growth during maturation [17,18]. Since the increase in CFA is an early event in this model, it could play an important role in nerve dysfunction. Preventing the increase in CFA may contribute to prevent the impairment of nerve dysfunction. Therefore the aim of the present study was to test over 3 months the effect of cérivastatin on peripheral CFA and peripheral nerve function in this model. If cerivastatin prevents both alterations, this may stand in favor of an action on microcirculatory endothelium dysfunction, presumably independent of lipid-lowering effects, and in favor of the role of CFA in nerve function impairment. To measure CFA we used a non-invasive isotopic method which makes possible to follow in vivo this parameter in diabetic rats and to evaluate the effects of pharmacological agents over several months [19].

2. Materials and methods

2.1. Animals

Diabetes was induced on 108 male rats born in the laboratory by intraperitoneal injection of STZ at a dose of 70 mg/kg in citrate buffer (pH 4.5) when these rats were aged 5 days. Fifty-three male Wistar rats aged 40 days were alive at the time of weaning. The animals did not receive any hypoglycemic agent during the trial. The study began when the rats reached a mean age of 3 months and ended when they were aged 7 months. Twenty normal male Wistar rats served as untreated controls (CU).

2.2. Experimental protocol

At the time of weaning, 40 diabetic rats were randomized in two groups to receive either cérivastatin (diabetic treated group, DT) or no treatment (diabetic untreated group, DU). Four diabetic rats, two of each group were tested on every investigation day.

The isotopic test was repeated three times:

- at a mean age of 3 months, before any treatment (time 0);
- then cérivastatin was administered to DT rats at the daily dose of 1 mg/kg by oral gavage during 2 months before the second test (time 1);
- after a 15-day wash-out period, cérivastatin was administered to DT rats at a dosage of 0.2 mg/kg per 24 h by oral gavage during 1 month until the third test (time 2).
Nerve electrophysiological measurements were repeated twice, at time 0 and time 2.

The same investigations were performed in untreated control rats.

Blood glucose was periodically monitored with a strip (One Touch II; Johnson–Johnon, Milpitas, CA) and before each test.

At each time, CFA test and electrophysiological measurements were performed in anesthetized animals. The animal was placed on a homothermic operating table and its central temperature was checked with a temperature sensing probe inserted rectally and maintained constant: 37°C ± 0.5°C during investigations.

This study was carried out according to the guide for the care and use of laboratory animals published by the US National Institute of Health (publications no. 85-23, revised 1996).

2.3. CFA test

Rats were anaesthetized with inhalation of mixed 1% fluothane and oxygen. Human serum albumin was labeled with 99 m Technetium and 37 MBq in 0.2 ml was injected intravenously into the tail. The animals were positioned prone. Radioactivity was measured on the two hindquarters with collimated lNa crystal activated with Tl (51 mm × 51 mm) located at 1 cm of the limb. A multichannel analyzer (4096 channels), with a time acquisition of 200 ms per channel, was used to obtain quantitative data. Acquisition was started at the steady state demonstrated by a constant radioactive curve achieved about 15 min after the albumin injection. The total number of counts per channel varied from 2000 to 8000 ips. The mean background was 4 counts per channel. A venous compression with a tourniquet (weight of 100 g) was applied on one limb 2.5 min after the beginning of measurement and exerted for 6 min. After removing the tourniquet, radioactivity was still measured during 5.5 min.

As previously shown in normal and diabetic rats [19], the radioactivity curve was characterized by three phases: (1) basal steady state (X); (2) an increase during venous compression leading to a maximal plateau (Y); (3) after removal of the tourniquet, a rapid decrease of radioactivity returning to a mean value (Z) which was close to the basal value in normal animals. Albumin retention (AR) was calculated as an index of CFA by using the formula:

\[ AR(\%) = \frac{[(Z - X)/(Y - X)]}{X} \times 100. \]

The last part of radioactivity curve recorded after the removal of the tourniquet was also analyzed by the Fast Fourier Transform as previously described [20]. The time-function curve was transformed into a spectrum of peaks of discrete frequencies: high frequency from 1250 to 75 mHz and low frequency from 75 to 4 mHz. The ratio of the amplitudes of the low and high frequency peaks (LF/HF) was calculated and may be considered as an index of lymphatic function uptake [20].

2.4. Nerve electrophysiological measurements

2.4.1. Motor nerve conduction velocity (MNCV)

The motor potential was recorded in the muscles of the first interosseus space (anodic electrode near the fourth toe) after a proximal monopolar cathodic stimulation at the sciatic notch (anodic electrode far away) and distal posterior tibialis nerve stimulation behind the internal malleolus (anodic electrode was behind the external malleolus). A ground electrode was between the proximal and distal stimulations.

Just supramaximal stimuli (0.1 ms, 13 mA) were delivered from an ESAOTE stimulator. The muscle action potential was suitably amplified and displayed on the screen.

Two or three consecutive action potentials from the two stimulating points were superimposed and latencies of the potential were measured. MNCV was calculated using estimated length of the nerve between the two stimulating electrodes measured on the skin.

2.4.2. Sensory nerve measurements

Recordings of the nerve potentials were made from the sciatic notch (anodic electrode far away) after bipolar stimulation of the external saphenous nerve.

The stimulating electrodes were inserted through the skin below the external malleolus to be along the nerve side.

Supramaximal stimuli were delivered at a 1.7 per s rate. One hundred responses were averaged. The average response was triphasic, positive, negative, and positive. The first positive peak latency was measured. The length between stimulating and recording electrodes was measured on the skin, and the sensory nerve conduction velocity (SNCV) was calculated, and the duration of the sensory nerve action potential (SNAP) was measured.

The reproducibility of nerve electrophysiological measurements was tested by performing these measurements twice on 12 control rats at a 30-min interval. After the first measurement, the rats remained in the same position on the homothermic operating table, and the electrodes were removed from the lower limbs and fixed to the back until the second measurement. The coefficients of correlation between the two measurements were 0.87, 0.90 and 0.95 (P < 0.001 for all) for MNCV, the latency of the sensory nerve potential and SNAP duration, respectively.

2.4.3. Lipid measurements

Serum total cholesterol and triglycerides were determined in untreated and cerivastatin-treated diabetic rats at time 2.

2.5. Statistical analyses

Data are given as mean ± S.E.M. for all quantitative parameters. Comparisons between groups were carried out by ANOVA or Kruskal-Wallis tests. Comparisons between paired data were also calculated by ANOVA. The statistical tests were performed using SPSS software (SPSS, Chicago, IL).
3. Results

There were 20 rats in each group. Mean body weights at the investigation times are shown on Table 1.

After the first series of investigations (time 0), the treatment with cerivastatin was started at the dose of 1 mg/kg per 24 h for DT rats. After a treatment period of 61 ± 10 days, there was a 15-day wash-out.

At time 1, a second series of investigations was performed on 13 treated rats with a mean age of 150 ± 4 days and 17 untreated rats aged 155 ± 6 days. Body weight and age were not significantly different in the two groups (Table 1). Some DT rats had muscle weakness with abnormal electromyographic patterns.

After the 15-day wash-out period, cerivastatin was administered at a daily dosage of 0.2 mg/kg during 39 ± 4 days. The disappearance of muscle toxic effects was observed.

At time 2, for the third series of investigations, there were 11 rats in the group DT with a mean age of 202 ± 6 days and 15 rats in the group DU with a mean age of 200 ± 5 days (Table 1).

3.1. Blood glucose and lipid measurements

At time 0, time 1 and time 2, blood glucose was not significantly different in the two diabetic groups. But, at each

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Age (days)</th>
<th>Body weight (g)</th>
<th>Blood glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>20</td>
<td>82 ± 6</td>
<td>352 ± 45</td>
<td>16.5 ± 1.5</td>
</tr>
<tr>
<td>DU</td>
<td>20</td>
<td>85 ± 5</td>
<td>360 ± 40</td>
<td>18.4 ± 1.2</td>
</tr>
<tr>
<td>CU</td>
<td>20</td>
<td>84 ± 5</td>
<td>420 ± 30</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td><strong>Time 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>13</td>
<td>150 ± 4</td>
<td>400 ± 26</td>
<td>17.2 ± 1.4</td>
</tr>
<tr>
<td>DU</td>
<td>17</td>
<td>155 ± 6</td>
<td>440 ± 22</td>
<td>20.6 ± 1.3</td>
</tr>
<tr>
<td>CU</td>
<td>18</td>
<td>160 ± 7</td>
<td>480 ± 35</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td><strong>Time 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>11</td>
<td>202 ± 6</td>
<td>466 ± 18</td>
<td>17.8 ± 1.3</td>
</tr>
<tr>
<td>DU</td>
<td>15</td>
<td>200 ± 5</td>
<td>471 ± 28</td>
<td>21.6 ± 1.0</td>
</tr>
<tr>
<td>CU</td>
<td>16</td>
<td>205 ± 6</td>
<td>520 ± 30</td>
<td>6.0 ± 0.7</td>
</tr>
</tbody>
</table>

Serum lipid levels were only measured at time 2. Total cholesterol was significantly lower in cerivastatin-treated diabetic rats than in untreated rats: 1.65 ± 0.13 g/l vs. 2.05 ± 0.14 g/l (P < 0.05) whereas triglyceride levels did not differ significantly in the two groups: 1.01 ± 0.09 g/l and 1.13 ± 0.05 g/l, respectively.

3.2. CFA

At time 0, AR was not significantly different in the two diabetic groups but significantly higher in both groups than in group CU: DU vs. CU and DT vs. CU (Fig. 1). AR increased significantly from time 0 to time 1 in group DU (P < 0.001) whereas AR did not change in groups DT and CU during the same period.

At time 1, AR was significantly higher in group DU than in groups DT and CU. From time 1 to time 2, AR increased significantly in group DU and did not change in groups DT and CU. At time 2, AR was again significantly higher in group DU than in group DT but AR did not differ significantly in groups DT and CU.

At time 0, LF/HF ratio was not significantly different in the three groups (Fig. 2). In group DU, LF/HF increased slightly from time 0 to time 1 and markedly from time 1 to time 2 whereas it did not change significantly in groups DT and CU.

At time 2, LF/HF was significantly higher in group DU than in groups DT and CU (Fig. 2).

3.3. MNCV

At time 0, MNCV was not significantly different in the three groups. At time 2, MNCV did not differ significantly between the two diabetic groups but MNCV was significantly lower only in group DU as compared to group CU (P = 0.002) (Fig. 3).

3.4. SNCV

At time 0, SNCV was not significantly different in the three groups. At time 2, there was no significant difference between
the two diabetic groups but SNCV was significantly lower only in group DU as compared to group CU ($P = 0.03$) (Fig. 4).

3.5. Duration of the sensory nerve potential

At time 0, SNAP duration was not significantly different in the three groups. At time 2, as compared to group CU, SNAP duration was significantly longer only in group DU ($P < 0.01$) (Fig. 5).

4. Discussion

The current study confirms our previous observation that peripheral interstitial AR, i.e. CFA, measured by a non-

Fig. 2. Changes in lymphatic index (LF/HF). ***: $P < 0.001$ group DU vs. group CU. †: $P < 0.007$, group DU vs. group DT.

Fig. 3. MNCV. * $P < 0.002$ group DU vs. group CU.

Fig. 4. SNCV. * $P < 0.05$ group DU vs. group CU.
invasive isotopic test increases after a few weeks of diabetes in rats with early-induced diabetes. It also confirms that a pharmacological agent may prevent such a disorder as we previously reported with an anthocyanoside extract [19]. It shows for the first time that cerivastatin may prevent significantly this disorder and strongly suggests that this agent exerts a specific microcirculatory effect. Diabetic rats were first treated by a high dose (1 mg/kg per day) of cerivastatin [21] to get a significant effect in a short delay on CFA. The beneficial effect on CFA was still found at a lower dose (0.2 mg/kg per day) of cerivastatin which maintained CFA at a low level. This result was observed after 1 month of treatment separated from the first period by a 15-day wash-out period, which is likely to exclude a carry-on effect.

Regarding the second index provided by the isotopic test, LF/HF may be considered as an index of lymphatic function uptake of interstitial albumin [20]. This index deteriorated in untreated diabetic rats later than AR index, as we previously described [19]. This phenomenon suggests that lymphatic dysfunction results from a saturation of lymph pumps [8]. Cerivastatin prevented efficiently this alteration, probably by reducing CFA.

Taken together these data strongly suggest a specific effect of cerivastatin on microcirculatory dysfunction associated with diabetes. The decrease in CFA is in agreement with the recent reports of a decrease in albuminuria and permeability of various tissues in diabetic rats treated by cerivastatin, simvastatin, or rosuvastatin, respectively [13,14,22] although the effects on vascular permeability did not seem to be homogeneous possibly due to some pitfalls in the method used or to a too short treatment [14]. Therefore the effects of statins on capillary permeability are likely to be ubiquitous.

There is no data available in favor of the role of lipid disorders, in particular an excess of LDL-cholesterol or an increased oxidation rate of LDL, on microcirculatory function. Total cholesterol was significantly reduced in DT group, which may not exclude a relation between the microcirculatory effect of cerivastatin and lipid improvement. However the beneficial effects of statins on diabetic nephropathy and on retinal permeability have been shown to be independent of their cholesterol-lowering effect [13,22]. Several factors might account for the protective effect of statins against vascular permeability including the attenuation of leukocyte–endothelial cell interactions [13], a decrease in blood pressure as recently reported in hypertensive animal models [23,24], and effects against inflammatory processes [25,26].

Regarding nerve function, the present results confirm that in rats with early-induced diabetes NCVs are reduced and the duration of sensory nerve potential is longer at 6 months of diabetes and not at 3 months as compared with control rats, which is in agreement with nerve protection during maturation [16]. The lengthening of the duration of the sensory nerve potential accounts for the temporal dispersion of the fast nerve fibers [27]. In the animals treated by cerivastatin, motor and sensory conduction velocities did not differ significantly from control animals, and even the sensory potential duration was close to normal values (Fig. 5). Therefore the present data suggest that cerivastatin may prevent partly nerve changes that occur between 3 and 6 months, in rats with early-induced diabetes. Rosuvastatin has been tested in adult rats with STZ-induced diabetes for 6 weeks. After 2 weeks of rosuvastatin treatment, the deficit in NCV was corrected in a dose dependent manner [15]. Thus statins appear able to prevent nerve function deterioration in STZ-induced diabetes whatever diabetes is induced by weaning or in adult rats. As to the mechanism of statin effects on improving nerve function, a correction of nerve blood flow deficit has been shown with rosuvastatin [15]. Moreover, Nukada and Pollock [9] had proposed a key role for endoneurial edema as a consequence of blood flow deficit during ischemia on diabetic nerve
changes. This phenomenon has been recently investigated through experiments of ischemia and reperfusion in diabetic rats. There was evidence for an increase of endoneurial edema in diabetic rats versus controls, and interestingly after reperfusion morphological anomalies of myelinated fibers and persistent reduction of motor nerve amplitudes in diabetic rats and no in control rats [28,29]. These data and our current observation of a prevention of peripheral CFA increase and a partial prevention of nerve conduction anomalies by cerivastatin strongly suggest a major role for endoneurium swelling in nerve conduction alterations induced by diabetes and that the beneficial effects of statins on nerve conduction may result from a reduction of endoneurial edema.

As to skeletal muscle disorders which occurred in some rats during the first period of treatment with 1 mg/kg per day of cerivastatin, they are at variance with previous studies that did not report any muscle disorders in normal rats treated even by higher dose (5 mg/kg per day) of cerivastatin [30]. Neither mitochondrial injury nor a decrease in muscle ubiquitine levels seem to be the primary cause of skeletal muscle toxicity in cerivastatin-dosed rats [31], but the role of exercise has been suggested [32]. Many authors [33–35] have emphasized the deleterious potential effect of the combination of two lipid-lowering drugs and the influence of another disease which can potentiate the effect of two drugs. Diabetes is not known as an enhancing factor of such toxic effects. However metabolic changes or immune-mediated mechanisms associated with diabetes might induce negative effects on the detoxicating role of the liver.

In conclusion the present study shows for the first time that a statin may prevent the increase in peripheral CFA associated with diabetes. Together with previous reports, this data strongly suggests that statin effects on capillary permeability are ubiquitous and represent another feature of the protective effect of statins on the endothelium. Peripheral nerve changes were also prevented partly by cerivastatin. The beneficial effect on microcirculation may reduce endoneurium swelling and thus contribute to the prevention of diabetes-induced peripheral nerve function impairment. The underlying mechanisms are likely to be independent of a lipid-lowering effect, but their clarification needs further investigation.

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