Effect of cerivastatin on proteinuria and urinary podocytes in patients with chronic glomerulonephritis

Tsukasa Nakamura1, Chifuyu Ushiyama1, Kaoru Hirokawa1, Shiwori Osada2, Teruo Inoue3, Noriaki Shimada4 and Hikaru Koide4

1Department of Medicine, Misato Junshin Hospital, Saitama, 2Department of Medicine, National Rehabilitation Center, Saitama, 3Department of Cardiology, Koshigaya Hospital, Dokkyo University School of Medicine, Saitama and 4Department of Medicine, Koto Hospital, Tokyo, Japan

Abstract
Background. We previously reported urinary podocytes to be a marker of glomerular injury. The aim of the present study was to determine whether cerivastatin, a newly developed, potent synthetic statin, affects proteinuria and urinary podocyte excretion in patients with chronic glomerulonephritis (CGN).

Methods. We randomly assigned 40 normotensive hypercholesterolemic patients with CGN to receive either cerivastatin 0.15 mg/day (n = 20) or placebo (n = 20). Subjects comprised 24 men and 16 women, with a mean age of 40.8 ± 14.4 years; 27 had IgA nephropathy and 13 had non-IgA proliferative glomerulonephritis. Treatment was continued for 6 months. Plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides, urinary protein excretion and the number of podocytes were measured before treatment and at 3 and 6 months after treatment.

Results. After 6 months, a significant reduction in total cholesterol (P < 0.001), LDL-cholesterol (P < 0.001) and triglycerides (P < 0.05), and a significant increase in HDL-cholesterol (P < 0.001) were observed in the group treated with cerivastatin. Urinary protein excretion decreased from 1.8 ± 0.6 to 0.8 ± 0.4 g/day, (P < 0.01) in this group, and urinary podocyte excretion decreased from 1.6 ± 0.6 to 0.9 ± 0.4 cells/ml (P < 0.01). However, placebo showed little effect on these lipid levels, urinary protein excretion and urinary podocyte excretion. The differences between the cerivastatin group and the placebo group were significant (cholesterol, P < 0.001; LDL-cholesterol, P < 0.001; triglycerides, P < 0.05; HDL-cholesterol, P < 0.001; urinary protein, P < 0.01; and urinary podocytes, P < 0.01).

Conclusion. Statins such as cerivastatin may be beneficial for restoration of injured podocytes in patients with CGN and hypercholesterolaemia.

Keywords: cerivastatin; glomerular epithelial cells; hypercholesterolaemia; podocalyxin

Introduction
There is increasing evidence to suggest that lipid abnormalities play a role in the progression of renal disease [1,2]. In patients with diabetic nephropathy, hyperlipidaemia has been associated negatively with the glomerular filtration rate [2], and simvastatin has been shown to reduce urinary albumin excretion [3]. Syrjanen et al. [1] reported hypertriglyceridaemia to be a risk factor for progression of IgA nephropathy. While the statins are effective for prevention of end-stage renal disease, the benefits are not fully understood [4]. Fried et al. [5] recently reviewed all the clinical data available and reported that lipid reduction had a beneficial effect on declining glomerular filtration. Cerivastatin is a novel, potent and highly selective inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which effectively reduces serum LDL-cholesterol concentrations when administered in very low doses [6].

One of the major cell types responsible for maintenance of the structure and function of the glomerular filter is the podocyte. Glomerular injury is usually associated with the leakage of protein across the filter into the urine and with the disappearance of podocyte foot processes, either locally or generally [7]. The most severe podocyte lesion occurs at the same time as detachment from the glomerular basement membrane, and the detached podocytes subsequently appear in the urine [8]. We have previously reported urinary podocytes to be a marker of the severity of acute
glomerular injury and a possible predictor of disease progression [8,9].

In the present study, we investigated the effect of cerivastatin on urinary protein and urinary podocyte excretion in proteinuric normotensive chronic glomerulonephritis (CGN) patients with hypercholesterolaemia.

**Subjects and methods**

**Patients**

Forty patients diagnosed histologically as having IgA nephropathy (n = 27) or diffuse mesangial proliferative glomerulonephritis without IgA deposition (n = 13), all with hypercholesterolaemia, were enrolled in the present study (24 men, 16 women; mean age 40.8 ± 14.4 years). All patients gave informed consent prior to participation in the study. Twenty hypercholesterolaemic patients without renal disease (12 men, 8 women; mean age 38.8 ± 12.2 years) served as controls. No patient had heart disease, collagen disease or diabetes mellitus. Hypertension >140/90 mmHg and renal dysfunction (serum creatinine >1.5 mg/dl or 24 h creatinine clearance <80 ml/min) were exclusion criteria. No previous immunosuppressive treatment, no steroids, no non-steroidal anti-inflammatory drugs, and no antihypertensive drugs were used by any subject before enrolment. However, antplatelet drugs such as dipyridamole or dilazep dihydrochloride had been administered to 20 CGN patients (10 IgA nephropathy patients and 10 patients with diffuse mesangial proliferative glomerulonephritis without IgA deposition). Dilazep dihydrochloride exerts its antplatelet activity by increasing the adenosine levels in the extracellular space fluid and it is also known to have a vasodilating activity [10]. These drugs were withdrawn 4 weeks before enrolment, as previously recommended [11]. Hyperlipidaemia was defined as follows: total cholesterol >200 mg/dl, LDL-cholesterol >160 mg/dl, HDL-cholesterol <35 mg/dl and or triglycerides >150 mg/dl [12]. Serum creatinine, blood urea nitrogen and urinary creatinine were determined by routine methods. Urinary protein levels were determined by the Lowry method. Twenty-four-hour urine samples were collected from all CGN patients.

The 40 patients with CGN and hypercholesterolaemia were randomly assigned to one of two groups: those treated with 0.15 mg/day cerivastatin (Takeda Chemical Industries Ltd, Osaka, Japan) (cerivastatin treatment group; 14 patients with IgA nephropathy and six patients with diffuse mesangial proliferative glomerulonephritis without IgA deposition) and those treated with placebo (placebo group; 13 patients with IgA nephropathy and seven patients with diffuse mesangial proliferative glomerulonephritis without IgA deposition) for 6 months. The 20 hypercholesterolaemic patients without renal disease were also divided into two groups (n = 10 in each group) treated with cerivastatin or placebo. At 3 and 6 months after the start of treatment, blood and urine were obtained for follow-up determination.

**Immunohistochemistry for quantification of urinary podocytes**

Freshly voided urine was collected for 5 consecutive days, and the number of urinary podocytes was counted daily. Urine specimens were processed within 30 min of voiding: 10 ml of fresh urine was centrifuged for 5 min at 700 g. The supernatant was aspirated, and the sediment was washed with 0.01 M phosphate buffer (phosphate-buffered saline (PBS)), pH 7.2. The sediment was then resuspended in 10 ml PBS, cytocentrifuged for 5 min at 700 g onto poly-1-lysine-coated microscope slides, and air-dried for at least 30 min. Slides were then fixed for 5 min in acetone at 4°C. Urine sediments on the slides were partitioned with a PAP pen (Dako, Tokyo, Japan) into six 1.0 x 1.0 cm² areas. After washing with PBS, each area was incubated for 60 min with 20 µl anti-human podocalyxin monoclonal antibody PHM-5 (Australian Monoclonal Development, Artamon, New South Wales, Australia) at a 1:200 dilution [8,9]. After another washing, the slides were incubated with fluorescein-isothiocyanate-labelled F(ab’)2 fragments of affinity-purified, anti-mouse IgG (Cappel ICN Biomedicals, Inc., Costa Mesa, CA, USA) as reported previously [8,9]. Slides were washed and examined by immunofluorescence microscopy. Nuclei of the cells were counterstained with ethidium bromide before mounting. Quantitative analysis was performed by three different pathologists in a blind fashion. The number of urinary podocytes is shown as cells per millilitre of urine. We reported previously that podocalyxin was present in the urine sediments of paediatric patients with glomerular disease in the form of cast, fine granules or whole cells [8]. In the present study we measured entire cells, not cell fragments, in the urine [9].

**Statistics**

Data are shown as mean ± standard deviation (SD). Results were analysed by two-way analysis of variance, and specific comparisons between groups were carried out by the two-tailed student’s t-test. Spearman’s rank correlation was used for the calculation of the coefficient of correlation. Statistical significance was determined at P < 0.05.

**Results**

Podocalyxin-positive cells were detected in the urine sediments of the 40 CGN patients with hypercholesterolaemia (2.2 ± 0.6 cells/ml) and were not detected in the 20 hypercholesterolaemic patients without renal disease. A significant positive correlation was observed between the number of urinary podocalyxin-positive cells and the urinary protein excretion level in CGN patients with hypercholesterolaemia (P < 0.01). Baseline systolic and diastolic pressures did not differ significantly between the cerivastatin-treatment group and the placebo group, and blood pressure did not change significantly in either the cerivastatin or placebo group. Baseline serum creatinine, blood urea nitrogen and 24-h creatinine clearance did not differ significantly between the groups, and none of these factors changed with treatment (Table 1). Baseline total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels did not differ significantly between groups, but significant decreases were observed in the cerivastatin-treatment group with respect to total cholesterol (3 months, P < 0.01; 6 months, P < 0.001), LDL-cholesterol (3 months, P < 0.01; 6 months, P < 0.001) and triglycerides (3 months, P < 0.05; 6 months,
P < 0.01), along with an increase in HDL-cholesterol (3 months, P < 0.01; 6 months, P < 0.001) (Figure 1). Baseline urinary protein excretion levels and the number of urinary podocalyxin-positive cells did not differ significantly between groups. Urinary protein excretion was reduced significantly in the patients treated with cerivastatin from 1.8 ± 0.6 g/day (baseline) to 1.2 ± 0.6 g/day at 3 months (P < 0.05) and 0.8 ± 0.4 g/day at 6 months (P < 0.01) (Figure 2). Urinary podocalyxin-positive cells were also reduced significantly in these patients from 1.6 ± 0.6 cells/ml (baseline) to 1.2 ± 0.5 cells/ml at 3 months (P < 0.05) and 0.9 ± 0.4 cells/ml at 6 months (P < 0.01) (Figure 2). However, placebo showed no significant effect on total cholesterol, LDL-cholesterol, triglyceride, HDL-cholesterol, urinary protein excretion and urinary podocyte excretion. The differences between the cerivastatin group and the placebo group were significant at 6 months (cholesterol, P < 0.001; LDL-cholesterol, P < 0.001; triglycerides, P < 0.05; HDL-cholesterol, P < 0.001; urinary protein, P < 0.01; urinary podocyte, P < 0.01).

In the 10 hypercholesterolemic patients without CGN who were given cerivastatin, total cholesterol,
LDL-cholesterol and triglyceride levels were reduced, and HDL-cholesterol level was increased. However, neither urinary protein excretion nor urinary podocalyxin-positive cell excretion was affected in these patients nor in those treated with placebo.

Discussion

Cerivastatin, a new statin, is of therapeutic value for the treatment of patients with hypercholesterolaemia [13]. In the present study we demonstrated that 0.15 mg cerivastatin is effective in CGN patients for ameliorating hyperlipidaemia and for reducing urinary protein excretion and urinary podocyte numbers.

It has been observed previously that glomerular sclerosis increases gradually in CGN including IgA nephropathy [1]. Analogous pathophysiological mechanisms have been proposed to operate in glomerulonephritis and atherosclerosis [14]. Impaired endothelium-dependent vasodilation plays a pivotal role in the pathogenesis of atherosclerosis. Endothelium-dependent vasodilation was reported to improve after 2 weeks of therapy with cerivastatin [15]. Monocytes play a determinant role in the progression of both glomerulonephritis and atherosclerosis. Monocyte infiltration and the expression of the adhesion molecule VCAM-1 were shown to be reduced by cerivastatin treatment [4]. Several studies have demonstrated the participation of monocytes in the onset and progression of CGN [4]. Monocytes play a role in pathological changes of chronic renal disease and have been associated with matrix accumulation and glomerulosclerosis: thus, cerivastatin may have a direct effect on these processes. Platelets are known to enhance monocyte activity, and cerivastatin reduces monocyte and platelet activities [16]. In the present study, the decrease in proteinuria by cerivastatin may be due, in part, to the decrease in monocyte and/or platelet activity. Recently, Buemi et al. [17] reported that another statin, fluvastatin, is effective in decreasing proteinuria in patients with IgA nephropathy. Our data coincides with theirs.

Podocytes form the filtration slit structure that prevents the escape of plasma protein from the glomerular circulation. Normal function of the filter requires the maintenance of the foot-process structures of the podocytes. Injury to the glomerulus is usually associated with the leakage of protein across the glomerular filter into the urine [7]. Recently, Meyer et al. [18] reported the number of podocytes per glomerulus to be the strongest predictor of renal disease progression among the morphological characteristics of the glomerulus.

The usefulness of quantifying detached podocytes is implicit, and we reported previously that it is possible to count the number of podocytes lost in the urine [8,9]. In the present study, we first demonstrated that cerivastatin reduced the urinary podocyte number in CGN patients. The precise mechanisms, however, are still unclear. We do not consider that urinary podocytes outweigh the importance of proteinuria; however, urinary podocyte number may be a useful laboratory marker for estimating the severity of glomerular injury, particularly active glomerular changes in CGN. Glomerular epithelial protein 1 (GLEPP1) was identified and cloned in a search of podocyte-specific proteins that play a role in regulating podocyte structure and function [7,19], and a reduction in GLEPP1 has been observed in the course of glomerular disease [19]. Moll et al. [20] recently reported complement receptor 1 (CR1) down-regulation as a possible marker of podocyte injury in glomerulonephritis. Urinary podocyte expression of both
GLEPP1 and CR1 should be investigated in relation to cerivastatin treatment.

In summary, cerivastatin reduced urinary protein excretion and urinary podocyte excretion in normotensive hypercholesterolemic CGN patients. Our data suggest that statins such as cerivastatin may be effective for repairing glomerular injury in cases of CGN.

Acknowledgements. We thank Ms Yukiko Suzuki, Ms Kazue Isaka and Mr Jun Kudoh (Renal Unit, Misato Junshin Hospital, Saitama, Japan) for their technical assistance.

References


Received for publication: 12.7.01
Accepted in revised form: 12.12.01