Danaparoid Sodium Lowers Proteinuria in Diabetic Nephropathy

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Abstract. Diabetic nephropathy is a progressive renal disease with thickening of the glomerular basement membrane and mesangial expansion and proliferation as histological hallmarks. The presence of the glycosaminoglycan (GAG) side chains of heparan sulfate proteoglycan, an important constituent of the glomerular basement membrane, is decreased in diabetic nephropathy proportionally to the degree of proteinuria. Danaparoid sodium is a mixture of sulfated glycosaminoglycans consisting mainly of heparan sulfate. The study presented here involved performing a randomized placebo-controlled crossover study with danaparoid sodium in diabetic patients with overt proteinuria. The aim of the study was to evaluate the effect on proteinuria and safety/tolerability. Nine patients completed the study, without major side effects; the crossover study consisted of two 6-wk periods of treatment with 750 anti-Xa units danaparoid sodium subcutaneously once-daily or placebo. Following danaparoid sodium, significant declines of both albuminuria and proteinuria were found. After danaparoid sodium, the albumin excretion ratio standardized for urinary creatinine reduced with 17% in comparison with an increase of 23% after placebo (95% confidence interval of the difference, −75.9–3.9%; P = 0.03). The percentage change of the urinary protein excretion corrected for urinary creatinine differed at 8 wk significantly between both treatment arms (P = 0.001). Additional parameters for safety as hematological, hemostasis, biochemical parameters, and fundusphotography did not show any clinically significant difference for both groups. Only two patients had minor skin hematomas at the injection site while using danaparoid sodium.

In conclusion, the supplementation was found to be feasible and was not associated with side effects. A significant decline of proteinuria was found. More prospective dose-finding and long-term studies must be performed to see whether danaparoid sodium could not only induce a reduction of proteinuria but also halt the progression of renal disease. (J Am Soc Nephrol 8: 456–462, 1997)
Danaparoid sodium is a new, commercially available, heparinoid. It is a mixture of sulfated GAGs, consisting mainly of HS with a small subfraction, which is highly sulfated. It is used both for the prevention and treatment of venous thrombembolism, where it has been shown to be efficacious and relatively safe. It functions by augmenting the inhibitory action of antithrombin on activated clotting factors Xa and IIa. The high ratio of anti-Xa/anti-IIa activity is thought to be responsible for a good efficacy/safety profile. It is seldom associated with heparin-induced thrombocytopenia. Clearance takes place mainly by the kidneys (21-23). Therefore, and because danaparoid sodium consists to a large extent of heparan sulfate, we decided to study the effect of danaparoid sodium on proteinuria in type I diabetic patients.

Materials and Methods

Design

The study was designed as a phase II, randomized, double-blind, placebo-controlled, crossover study. The medical ethical committee of the Leiden University Hospital had approved the protocol of the study, and all patients gave informed consent. Eligible patients were randomly allocated to one of the two treatment groups: 750 anti-Xa units danaparoid sodium (Orgaran®, NV Organon, Oss, the Netherlands), subcutaneously (SC) administered once daily for 6 wk; a wash-out period of at least 4 wk; placebo (saline with sulfite), SC administered once-daily for 6 wk, or the same scheme in the opposite direction. Medical and laboratory assessments were performed every 2 wk (0 = baseline; 2, 4, 6, and 8 wk after start of the respective treatments).

Patients

The study population consisted of nine patients who had nephropathy due to DBM and fulfilled the following selection criteria: they had to be >18 years old; they had to have insulin-dependent diabetes mellitus type I (DM I) with macroalbuminuria (AER >300 mg/24 h) because of diabetic nephropathy and not because of other renal disease; and they had to give informed consent. The use of angiotensin I converting enzyme (ACE)-inhibitors and other antihypertensive medications was only allowed if given at a stable dosage at least 6 wk prior to start of the study.

Patients complying with the inclusion criteria were eligible if none of the following exclusion criteria were present: pregnancy or wish to become pregnant; hemorrhagic diathesis; use of medications known to interfere with hemostasis and/or platelet function; use of corticosteroids; uncontrolled hypertension; active proliferative retinopathy; history of heparin-induced thrombocytopenia; severe hepatic failure; creatinine clearance of <40 mL/min; hypersensitivity to sulfite.

Medication

Danaparoid sodium is a mixture of sulfated GAGs, consisting of 84% heparan sulfate (HS), 12% dermatan sulfate, and 4% chondroitin sulfate isolated from porcine intestinal mucosa. It has an average molecular weight of 4000-7000 d, a specific anti-Xa activity of 11.0-17.0 U/mg, and an anti-Xa/anti-IIa ratio of >22. The elimination half-life of anti-Xa activity is ~25 h. The half-life time is only affected by severe renal failure, as seen in patients on chronic hemodialysis. The placebo had the same appearance as the danaparoid sodium containing ampoules; it contained isotonic sodium chloride and sodium sulfate. Danaparoid sodium (0.6 ml, 750 anti-Xa units) and the placebo were administered once-daily by subcutaneous (SC) injection for 6 wk, followed by a wash-out period of at least 4 wk. The patients were trained to self-administer the study medication in an abdominal skinfold. The date and time of each injection was recorded daily by the patient on a list and checked by the investigator at the end of each treatment period, together with the number of returned unused ampoules. Protamine-containing insulin preparations were not allowed to be injected at the same site, and study medication was not allowed to be mixed with insulin.

Efficacy Assessments

In diabetic nephropathy, both proteinuria and macroalbuminuria are associated with a steady decline of renal function. Taking the relative short duration of treatment into account, we decided to use proteinuria and albuminuria as surrogate markers. These parameters were expressed per mmol of creatinine to correct for incomplete urinary collections. The main parameters for efficacy were urinary albumin excretion rate (AER) and urinary protein excretion rate (PER) corrected for urinary creatinine excretion rate. Therefore, total protein, albumin, and creatinine contents in 2 × 24-h urine samples (collected by the subject at home) were assessed. Baseline measurements were taken before both treatment periods. At 0, 4, 6, and 8 wk, AER, PER, and creatinine clearance were measured twice, and the means were compared with baseline (0 wk).

Safety Assessments

A general medical history (including duration of patients' DM I), height, weight, blood pressure, and heart rate after 3 min in sitting position were taken, and a medical examination was performed. Fundusphotography (including staging of observed retinopathy) was performed before the inclusion and at the end of the study period. Sodium, potassium, urea, creatinine, glucose, total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, γGT, HbA1c, hemoglobin, hematocrit, leukocytes, thrombocytes, prothrombin time, antithrombin III, activated partial thromboplastin time (APTT), and plasma anti-Xa activity were tested regularly as safety and tolerability parameters.

Laboratory Assessments

Urinary albumin was assessed using immunonephelometry on an autoanalyzer (Array Protein system, Beckman Instruments, Brea, CA) with specific antibodies. Urinary protein, serum sodium, potassium, urea, creatinine, protein, albumin, SGOT, SGPT, alkaline phosphatase, and γGT levels were assessed on a Hitachi 747 or 911 autoanalyzer (Boehringer, Mannheim, Germany). The Technicon H-1 system (Bayer Diagnostics, München, Germany) was used for the analysis of hematological parameters. Prothrombin time was determined using the Thromborel S reagent (Behringwerke AG, Marburg, Germany), and APTT was determined using the Cephotest reagent (Nyegaard, Oslo, Norway), both on an Electra 1000C coagulometer (MLA, Pleasantville, NY). Antithrombin and anti-Xa activity were assessed on an ACL 200 (Instrumentation Laboratory, Milan, Italy) with Coamatic Antithrombin and Coatest LMWHeparin as reagents (Chromogenix, Mölndal, Sweden). HbA1c was assessed on high-performance liquid chromatography (HPLC) after hemolysis.
Table 1. Demographic data

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>DM (y)</th>
<th>ACEi</th>
<th>BMI (kg/m²)</th>
<th>MAP (mmHg)</th>
<th>HbAlc (%)</th>
<th>AER (mg/24 h)</th>
<th>AER/Creat (mg/mmol)</th>
<th>PER/Creat (mg/mmol)</th>
<th>CrCl (ml/min)</th>
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</thead>
<tbody>
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<td>119</td>
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<td>7.6</td>
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<td>250</td>
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<td>62</td>
</tr>
<tr>
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<td>7.4</td>
<td>968</td>
<td>63</td>
<td>97</td>
<td>127</td>
</tr>
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<td>M</td>
<td>No</td>
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<td>27.1</td>
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<td>10.8</td>
<td>715</td>
<td>95</td>
<td>103</td>
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<td>No</td>
<td>27.1</td>
<td>23.9</td>
<td>103</td>
<td>7.4</td>
<td>968</td>
<td>63</td>
<td>97</td>
<td>127</td>
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<tr>
<td>7</td>
<td>29</td>
<td>M</td>
<td>No</td>
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<td>23.9</td>
<td>103</td>
<td>9.8</td>
<td>912</td>
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<td>86</td>
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<td>23.9</td>
<td>103</td>
<td>9.8</td>
<td>912</td>
<td>64</td>
<td>86</td>
<td>141</td>
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<tr>
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<td>25</td>
<td>M</td>
<td>Yes</td>
<td>27.6</td>
<td>23.9</td>
<td>103</td>
<td>9.8</td>
<td>912</td>
<td>64</td>
<td>86</td>
<td>141</td>
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</tbody>
</table>

a DM, diabetes mellitus type I; ACEi, yes denotes the use of ACE inhibitor, and no denotes no ACE inhibition; BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure; AER/creat, urinary albumin excretion rate standardized for urinary creatinine excretion rate; PER/creat, urinary protein excretion rate standardized for urinary creatinine excretion rate; CrCl, creatinine clearance.

Statistical Analyses

The mean change of the primary and secondary parameters during the two treatment periods were analyzed using a mixed-model analysis of variance (ANOVA), with random patient factor to account for the correlations between the repeated measurement, and fixed treatment and time factors. In addition, single degree-of-freedom (paired t) tests were used to evaluate differences between and within the two treatment periods. Because the distribution of the primary parameters was extremely skewed, a logarithmic transformation was applied. Values (relative change) for AER/creat and PER/creat were calculated as follows:

\[
(y - x/x) \times 100 = \delta,
\]

where y is the nth week value for AER/creat or PER/creat and x is the 0 week value. Significance was accepted at the P-value level of 0.05. The calculations were performed with the SPSS package release 6.0 for Windows.

Results

Patients

Nine patients were enrolled in the study, and all completed both crossover treatment periods. Prior to the study, two patients were treated with lasercoagulation for proliferative retinopathy for ophthalmologic reasons only. Demographic data and characteristics are given in Table 1. In general, the HbAlc reflected an acceptable metabolic control of the diabetes. In contrast, the blood pressure was rather high for these patients. The albuminuria and proteinuria ranged from borderline "overt" (patient 8) to proteinuria in the nephrotic range (patient 3). Four patients were on ACE-inhibitors, and one of these also used amlodipine. Table 2 shows the primary parameters per group and treatment, Tables 3a and 3b show individual results.

HbAlc was 8.5% before the treatment period with danaparoid sodium and 8.3% before placebo (P = 0.4). No major changes were made for the insulin dosage during both periods. The baseline values of the MAP did not differ between the two groups (103 mmHg and 105 mmHg; P = 0.3). No major adverse events occurred. One patient needed antibiotic treatment during the placebo period because of sinusitis without fever. Subcutaneous injections were performed once daily dur-

Table 2. Creatinine clearance, blood pressure, AER/creatine, and PER/creatine before and after treatment with placebo and danaparoid sodium

<table>
<thead>
<tr>
<th></th>
<th>Danaparoid Sodium</th>
<th>Placebo</th>
<th></th>
<th></th>
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<th></th>
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<tr>
<td></td>
<td>0 wk</td>
<td>8 wk</td>
<td>P value</td>
<td>0 wk</td>
<td>8 wk</td>
<td>P value</td>
</tr>
<tr>
<td>CrCl (ml/min)</td>
<td>102.9 (75.5–130.4)</td>
<td>96.9 (68.4–125.4)</td>
<td>0.37</td>
<td>90.4 (74.0–106.8)</td>
<td>100.5 (69.7–131.4)</td>
<td>0.20</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>103.0 (100.6–105.4)</td>
<td>102.2 (98.5–106.0)</td>
<td>0.68</td>
<td>105.4 (98.9–111.8)</td>
<td>98.5 (92.7–104)</td>
<td>0.26</td>
</tr>
<tr>
<td>AER/creat (mg/mmol)</td>
<td>74.8 (42.7–131.2)</td>
<td>60.6 (33.1–110.9)</td>
<td>0.03</td>
<td>64.8 (36.7–114.7)</td>
<td>75.8 (37.2–154.3)</td>
<td>0.18</td>
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<tr>
<td>PER/creat (mg/mmol)</td>
<td>111.0 (65.5–187.9)</td>
<td>89.3 (51.9–153.8)</td>
<td>0.008</td>
<td>87.0 (51.9–146.0)</td>
<td>110.6 (57.4–213.1)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* All values are expressed as mean and (95% confidence interval of the mean), except for AER/creat and PER/creat, which are expressed as geometric mean (antilog 95% CI). CrCl, creatinine clearance; MAP, mean arterial pressure; AER/creat, urinary albumin excretion rate standardized for urinary creatinine excretion rate; PER/creat, urinary protein excretion rate standardized for urinary creatinine excretion rate. P values are obtained with paired t tests (within each treatment block).
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Table 3a. Individual results: AER/creat

<table>
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<tr>
<th>Patient No.</th>
<th>R</th>
<th>Danaparoid Sodium</th>
<th>0 wk</th>
<th>4 (δ) wk</th>
<th>6 (δ) wk</th>
<th>8 (δ) wk</th>
<th>Placebo</th>
<th>0 wk</th>
<th>4 (δ) wk</th>
<th>6 (δ) wk</th>
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<tr>
<td>1</td>
<td>D</td>
<td>64</td>
<td>83</td>
<td>(+29%)</td>
<td>75</td>
<td>(+17%)</td>
<td>53</td>
<td>(-18%)</td>
<td>78</td>
<td>63</td>
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</tr>
<tr>
<td>2</td>
<td>P</td>
<td>133</td>
<td>114</td>
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<td>111</td>
<td>(-17%)</td>
<td>107</td>
<td>(-19%)</td>
<td>90</td>
<td>74</td>
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<td>P</td>
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<td>(+19%)</td>
<td>77</td>
<td>(+3%)</td>
<td>63</td>
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Table 3b. Individual results: PER/creat

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<th>6 (δ) wk</th>
<th>8 (δ) wk</th>
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*Note. δ values (relative change) for AER/creat and PER/creat were calculated as follows: (y - x/x*100%) = δ, where y is the nth week value for AER/creat or PER/creat and x is the 0 wk value. R (randomization) denotes whether danaparoid sodium (D) or placebo (P) was the first treatment block; AER/creat (and PER/creat), urinary albumin (protein) excretion rate standardized for urinary creatinine excretion rate; nd, no data.

Efficacy Parameters

A significant decline of both albuminuria and proteinuria was found after treatment with danaparoid sodium compared with baseline and with the placebo treatment. Figure 1 shows the percentage reduction of albuminuria for both groups. Compared with baseline, the AER/creatinine at the end of the treatment period was -17% for the danaparoid sodium group and +23% for the placebo group (95% confidence interval (CI) of the difference -75.9 to -3.9, P = 0.03). Even after omitting the outlier of 126% increase during placebo, a significant difference was found (P < 0.05). The protein excretion rate standardized for creatinine excretion showed a decrease of 18% in the danaparoid sodium group, whereas an increase of 40% was seen in the placebo group (95% CI of the difference -115.8 to -1.0, P < 0.05). Figure 2 shows the mean percentage change of the urinary protein/creatinine ratios during both treatment regimens. The difference of the overall change pattern in both treatment groups was highly significant (P =...
dusphotography did not show hemorrhages, and no progression of retinopathy was found in any of the patients. Anti-Xa values were detectable only once in two patients (respectively, 0.12 and 0.10 U/mL; lower limit of detection <0.10 U/mL); this was not accompanied by any change of coagulation test results (APTT). No changes occurred for hematological parameters, and values within each patient during both treatment periods remained about the same for hemoglobin, leukocytes and thrombocytes. Liver enzymes (including alkaline phosphatase) did not change, either.

Safety Parameters

No changes in visual acuity occurred during treatment. Fundusphotography did not show hemorrhages, and no progression of retinopathy was found in any of the patients. Anti-Xa values were detectable only once in two patients (respectively, 0.12 and 0.10 U/mL; lower limit of detection <0.10 U/mL); this was not accompanied by any change of coagulation test results (APTT). No changes occurred for hematological parameters, and values within each patient during both treatment periods remained about the same for hemoglobin, leukocytes and thrombocytes. Liver enzymes (including alkaline phosphatase) did not change, either.

Discussion

Optimizing the treatment of DM I with the goal of slowing down the progression of vascular and renal damage has been the subject of many studies. In the past, intensified insulin treatment, antihypertensive treatment in general, and especially ACE inhibition have been shown to slow the progression of diabetic nephropathy (24–27). We decided to evaluate in a clinical setting the applicability of proteoglycans as an additional treatment modality. All of our patients had macroalbuminuria, and they had, therefore, passed the point of reversibility of this specific renal disease. The Danish group has already shown a benefit of LMWH and heparin for microalbuminuric patients (19). A previous study with enoxaparin in macroalbuminuric patients from our unit failed to demonstrate a statistically significant difference between placebo and LMWH (20), although a significant decrease in proteinuria was seen in the treated group when pretreatment values were taken into consideration. The difference between placebo and LMWH may not have been found because of the small number of patients studied and the short duration of the study. In the study presented here, we observed a clear reduction for the AER and PER after treatment with danaparoid sodium for 6 wk. Only one woman participated in this study (with a good response on therapy); we acknowledge more women need to be studied before our results may be applied to both sexes. Although not clinically or statistically significant, MAP showed a trend to decline in the placebo group. This might be explained by regression to the mean. We found no effects on blood pressure during danaparoid sodium treatment.

The most important result of our study is that the goal to reduce AER and PER by HS therapy may be feasible. The favorable chemical and clinical profile of danaparoid sodium in comparison to heparin and LMWHs made us decide to study this medication (28). Heparin-induced thrombocytopenia and thrombosis is a feared clinical picture with a dismal outcome. Only danaparoid sodium in comparison to heparin and LMWHs is associated with a very low incidence of this complication (28). Osteoporosis is another drawback associated with long-term heparin use. Limited experience with LMWHs showed a more favorable outcome in this respect (29,30).

Heparins and HS both exert anti-inflammatory actions as well as antiproliferative effects. An excellent short review on this topic was recently published by Wardle (31). Heparin prevents basic fibroblast growth factor induced proliferation and may also stop smooth muscle cell proliferation by releasing transforming growth factor β from its binding protein (32,33). It also inhibits the synthesis and release of endothelin-I by endothelial cells (34). The negative charge at the N position is thought to be required for the antiproliferative action (35).

Danaparoid sodium is not a LMWH but a heparinoid. It consists of 84% HS. Only a small fraction (4%-5%) of this HS is highly sulfated and is considered to be important for the anti-Xa activity. The fraction of HS with a low affinity for antithrombin III does not affect coagulation factors Xa and Xa but contributes substantially to the antithrombotic activity, probably through an effect on endothelial cells (23). The dose-related response to danaparoid sodium remains gradual and linear over a wide range, which may contribute to its safety. It has a 100% bioavailability after subcutaneous administration. Studies in humans have been using heparin or LMWH. We thought that danaparoid sodium could have a more favorable
benefit/risk profile in case of long-term prescription in comparison to LMWH. The theoretical arguments of a lower sulfate content and, hence, less effect on proteinuria were not validated by our findings. Whether only the 4%-5% high-affinity HS fraction has been of relevance for the antiproteinuric effect or the low sulfated fraction has been of a complementary benefit has yet to be established. Based on the half-life time of danaparoid sodium (25 h for the anti-Xa activity), a 4-wk wash-out period was thought—prior to the study—to be reasonable and long enough. The results and especially the increase of proteinuria during the placebo period suggest in retrospect that this increase might, in part, be explained by a carry-over effect. However, in the Danish study, the LMWH and the placebo groups also showed 3 months after the study an increase of albuminuria of 30% and 125% relative change, respectively (19). For further crossover studies, not only the drug half-life time, but also an additional lag-time because of the mediated effects of danaparoid sodium should be taken in consideration to prevent this putative confounding effect.

Our results are in agreement with previously reported results of proteoglycans on proteinuria in diabetic nephropathy (19,20). This short-term study demonstrates, as already seen in animal models, that proteoglycans can exert a reversible effect also at a late stage of nephropathy (18). Because we have not taken any renal biopsies to substantiate the level of changes on histological examinations, we can only hypothesize about the mechanism of action. As danaparoid sodium is mainly cleared by the kidneys, both replacement of "faulty" HS molecules in the GBM as well, as a beneficial effect on smooth muscle cell and mesangial cell proliferation, could have accounted for the clinically observed effects. In previous studies using sulfated glycosaminoglycans, no differences were found for glomerular filtration rate, renal plasma flow, and filtration fraction (19,20,36). This suggests that the antiproteinuric effect of HS is not related to altered renal hemodynamics.

In conclusion, the once-daily SC administration of 750 anti-Xa u of danaparoid sodium resulted in a significant reduction of proteinuria in patients with overt diabetic nephropathy. More clinical studies are needed to confirm and extend our findings. A dose-finding study should also be performed. It is obvious that only long-term studies will prove that the reduction of proteinuria will be associated with a slower decline or even maintenance of renal function in diabetic nephropathy.

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