Originals

Cyclosporin reduces renal prostanoid excretion in type 1 diabetic patients

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Abstract. Prostacyclin and thromboxane A 2 are important regulators of kidney blood flow. To examine whether changes in their metabolism could be involved in the nephrotoxicity of cyclosporin, we determined urinary excretion of 6-keto PGF 1α and dinor-6-keto PGF 1α (prostacyclin metabolites) and dinor-TxB 2 (thromboxane metabolite) in five newly diagnosed type 1 diabetic patients during and after stopping cyclosporin therapy. In the resting state, cyclosporin had no effect on prostanoid excretion. In response to exercise, urinary excretion of 6-keto PGF 1α was reduced by 50% (P<0.02), dinor-6-keto PGF 1α by 15% (P<0.05) and dinor-TxB 2 by 45% (P<0.02), while albumin excretion increased 4.5-fold (P<0.05) during cyclosporin therapy. Simultaneously, there was a rise in serum creatinine concentration, and renal biopsy specimens obtained from three patients showed periglomerular and interstitial fibrosis and tubular atrophy. After the discontinuation of cyclosporin therapy, serum creatinine concentrations returned to normal, histological changes improved and there was an associated rise in urinary prostanoid excretion. These data suggest that a reduction in renal prostanoid synthesis by cyclosporin may diminish renal blood flow and function, and lead to histological changes in the kidney.

Key words: Cyclosporin – Diabetes – Prostanoids

Introduction

Studies both in diabetes-prone BB rats [1] and in newly diagnosed type 1 diabetic patients [2–4] have demonstrated that cyclosporin can either prevent the development of autoimmune diabetes [1] or significantly increase the incidence and duration of remissions when the treatment is started soon after the diagnosis [2–4]. These benefits, however, are shadowed by adverse effects of cyclosporin, especially by its nephrotoxicity [5, 6]. This is particularly a problem in diabetes, where the disease itself may cause nephropathy. The mechanisms of the cyclosporin-induced nephropathy are poorly known. It appears to be haemodynamically mediated and is related to renal vasospasm. Studies in the rat suggest that cyclosporin causes intrarenal vasospasm [7]. Isotope studies in patients with renal transplants have shown increased renal vascular resistance, which is corrected when the cyclosporin is replaced by azathioprine [8]. Although the kidney transplant is a single-kidney model, these data suggest the involvement of cyclosporin in renal vascular resistance. Prostaglandins, and especially vasodilatory prostacyclin and vasoconstrictory thromboxane A 2 have a central role in the regulation of renal blood flow [9]. In cultured human endothelial cells cyclosporin inhibits prostacyclin production [10]. If cyclosporin inhibits renal prostanoid production in vivo, this could interfere with the regulation of renal capillary blood flow, and lead to a reduction in renal blood flow and ischaemic changes in the kidney. The present study was undertaken to examine the effect of cyclosporin therapy on basal and exercise-stimulated [11–13] urinary excretion of metabolites of prostacyclin and thromboxane A 2 in patients with type 1 diabetes.

Patients and methods

Patients. We studied five male type 1 diabetic patients, age 23±3 years (mean±SEM), weight 66±3 kg on admission, height 177±2 cm. They were the patients at the Helsinki University Hospital participating in the Canadian/European Diabetes Study on the effects of cyclosporin (CyA) in newly diagnosed type 1 diabetes [4], and randomized to the group for cyclosporin therapy. Before admission, the duration of diabetic symptoms was less than 12 weeks. On admission, their blood glucose averaged 19.1±1.0 mmol/l, they had ketonuria and mild acidosis (pH 7.35±0.04, base excess −8.9±5.8 mmol/l). The potential risks and benefits of the study were explained to the patients before their voluntary consent to participate was obtained. The protocol was approved by the Ethical Committee of the Helsinki University Hospital.
Treatment. Insulin therapy was started on admission as usual and was adjusted according to blood glucose levels with a target concentration below 7 mmol/l in the fasting state and before meals, and below 9 mmol/l postprandially. Cyclosporin therapy was started on mean of 8 days (range 3–12 days) after the start of insulin treatment. The initial cyclosporin dose was 10 mg/kg per day orally in divided doses at 12-h intervals, and the dosage was adjusted to maintain 12-h trough concentrations in whole blood of 400–800 ng/ml. If the serum creatinine rose to more than 150% of baseline value, the cyclosporin dosage was reduced in 20% steps until the creatinine level returned to below this action limit [4]. The average daily cyclosporin maintenance dosage was 7.3 ± 0.5 mg/kg. The cumulative dose was 2943 ± 263 mg/kg by the time the first exercise test was done. The mean concentration of whole blood cyclosporin throughout the treatment period was 648 ± 29 ng/ml. None of the patients was hypertensive during the cyclosporin therapy.

Procedures. The patients were studied twice for resting and exercise-induced urinary prostanoid excretion. The first test was performed during CyA therapy, which had lasted for 13.5 ± 2.8 months, and the second test a mean of 4.4 ± 0.7 months later (1.5 ± 0.4 months after the discontinuation of CyA therapy). At the time of the first exercise test, four of the five patients were in remission (fasting blood glucose < 7 mmol/l, HbA1 normal, no insulin therapy), while the fifth patient was receiving 36 units of insulin daily in two divided doses. When the test was repeated after the discontinuation of cyclosporin, three patients were in remission and the other two were receiving 31 and 36 units of insulin per day, respectively, as two injections. The two tests were performed in a similar fashion as follows. For the resting baseline measurements, the patients were admitted to the hospital at 8 p.m. for an overnight (12 h) urine collection. At 8 a.m., in the fasting state and without the morning insulin injection, the bladder was emptied and an indwelling catheter was inserted in an antecubital vein for blood sampling. Thereafter, the patients started a 40 min cycle ergometer exercise at an intensity intended to raise the heart rate to 140 min⁻¹. The absolute exercise intensity was 690 ± 34 kpm/min, the same in both tests. The heart rate response to exercise, as determined at 10 min intervals, was similar during the first and second test (140 ± 3 min⁻¹ and 138 ± 2 min⁻¹, respectively). The patients emptied the bladder at the end of the exercise and 60 min later.

For prostanoid measurement, urine samples (10 ml) were first passed through Sep-Pak C18 Cartridges (Waters Associates, Milford, MA, USA) to absorb prostanoids. The cartridge was then evaporated to dryness, dissolved in water-acetoni-trile-acetic acid (69.95 : 30.0 : 0.05 v/v/v) and put through HPLC (Spectra Physic M-740, Santa Clara, CA, USA; Spherisorb 5 U ODS II column) to separate the stable metabolites of prostacyclin, 6-keto-prostaglandin F1α (6-keto) and 2,3-dinor-6-keto-prostaglandin F1α (6-keto-dinor) and that of thromboxane A2 (2,3-dinor-thromboxane B2, dinor-TxB2). The appropriate fractions were collected, extracted with ethyl acetate, evaporated to dryness, dissolved in phosphate-buffered saline and assayed for each prostanoid by radioimmunoassay [14]. The 6-keto antibody had a cross-reactivity of 21% with dinor-6-keto and thus we were able to use 6-keto antiserum and tracer to measure dinor-6-keto. For dinor-TxB2 measurement we used TxB2 tracer and antiserum, which had a cross-reactivity of 44% with dinor-TxB2 [14]. Urinary excretion of albumin was determined by immunoturbidimetry [15]. To facilitate urine collections, the patients were encouraged to drink mineral water during and after the exercise.

Results

Glycosylated hemoglobin concentration was elevated at the time of diagnosis, but had returned to normal by the time of the exercise tests (Table 1). During cyclosporin therapy, there was a transient increase in serum creatinine concentrations. During the exercise, plasma glucose levels remained unchanged, whereas blood lactate levels rose to a similar extent during both tests (Table 2). During the first exercise test, serum free insulin levels were slightly higher than during the second test; the concentrations remained unchanged during exercise (Table 2).

Fig. 1 shows urinary excretion of 6-keto PGF2α, dinor 6-keto PGF2α and dinor-TxB2 in the resting state, immediately after the exercise and during the recovery period, and after stopping cyclosporin therapy. Cyclosporin treatment reduced 6-keto excretion in the resting state. In response to exercise, 6-keto excretion was reduced by 50%, dinor 6-keto by 15% and dinor-TxB2 by 45% during cyclosporin therapy. In the recovery period, the excretion of the prostanoïd 6-keto and dinor-TxB2 were still reduced during cyclosporin treatment, whereas dinor-6-keto excretion was unchanged. When the urinary excretions were calculated as pg/min, there were no differences in the basal state, whereas the excretion of each of the prostanoïd metabolites was reduced during and after exercise during cyclosporin therapy (Fig. 1).

Cyclosporin therapy had no effect on urinary albumin excretion in the resting state. During exercise and recovery.

Table 1. Glycosylated hemoglobin and serum creatinine concentrations at the time of diagnosis and at the time of the two exercise tests

<table>
<thead>
<tr>
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<th>At diagnosis</th>
<th>Exercise test during cyclosporin</th>
<th>Exercise test after cyclosporin</th>
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</thead>
<tbody>
<tr>
<td>HbA1 (%)</td>
<td>11.9 ± 0.6</td>
<td>7.4 ± 0.7</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>87 ± 4</td>
<td>109 ± 5*</td>
<td>94 ± 2</td>
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</table>

* P<0.05 compared to value at diagnosis or after cyclosporin treatment assay [17]. During exercise, blood glucose was determined using the glucose oxidase method [18], blood lactate by an enzymatic method [19], and serum free insulin by radioimmunoassay after precipitation with polyethylene glycol [20]. In the three patients, who were in remission after 12 months of cyclosporin therapy, a percutaneous renal biopsy was performed to decide whether or not to continue cyclosporin. The duration of cyclosporin therapy was 14–15 months at the time of biopsy. In these three patients, the cyclosporin therapy was discontinued because of the histological changes observed in the renal biopsy specimens, while the therapy was also discontinued in the other two patients not in remission. In the patient with most severe changes on renal histology, a repeat biopsy was taken 9 months after discontinuation of cyclosporin therapy. At this time (24 months after diagnosis) the patient was still in full remission.

Statistical methods. Analysis of variance and Student's paired t-test were used as appropriate. Because of the logarithmic normal distribution of urinary albumin excretion, the albumin excretion were subjected to logarithmic transformation before statistical analysis. The results are given as mean ± SEM.