Time-Dependence of Urinary Neopterin, a Marker of Cellular Immune Activity

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Neopterin, a marker of cellular immune system activation, is produced by human macrophages after induction by interferon gamma (secreted by T-lymphocytes) and is eliminated mostly in urine. We have documented the circadian rhythm of urinary neopterin in five healthy young men (about 25 years old), using voidings collected during 48 h at fixed 4-h intervals. We repeated the experiment three times, one week apart. Neopterin was measured by high-performance liquid chromatography (HPLC). We clearly show a peak of the excretion of neopterin in the early morning (around 0630 hours ± 2 h), with total variability (peak—trough difference) reaching 51%. Neopterin is commonly assayed in urinary fractions, so it is imperative to use urine specimens collected at the same time of day—e.g., the first morning urines—to avoid misinterpretation in follow-up of patients.

Additional Keyphrases: circadian rhythm · diseases involving cellular immunity

Neopterin is a metabolite derived from guanosine triphosphate. On stimulation with interferon gamma secreted by T-lymphocytes, human macrophages release neopterin into the bloodstream (1). It is eliminated almost exclusively in urine, therefore urinary neopterin is considered to be a good reflection of its biological production. Neopterin has been found useful in the follow-up of diseases involving cellular immunity, e.g., cancer (2), AIDS (3), Crohn’s disease (4), and sarcoidosis (5), and in allograft recipients (6). Given recent descriptions of large circadian variations of lymphocytes (OKT3, OKT4, NK cells) (7), we wondered if there was also a rhythmicity in the urinary excretion of neopterin.

Subjects, Materials, and Methods

Subjects

Five young men, medical students, ages 25 (SD 2) years, volunteered for the study. They were considered healthy on routine clinical and laboratory examinations. None took any medication before or during the study, and all had a regular social routine, with lights turned off at 2300 hours (±1 h) and on at 0730 hours (±1 h). Unrestricted meals were taken at 0730, 1230, and 2000 hours (all ±1 h).

Protocol

Each subject collected his total urine each 4 h during 48 h, beginning at 2030 hours, and this procedure was performed three times, once a week during late February and March. The total protocol therefore yielded 180 specimens. After collection, specimens were promptly stored in darkness at −20 °C until assay.

Analytical Procedures

Urinary neopterin and creatinine were determined by reversed-phase HPLC as previously described (8). In brief: for HPLC we used an LC 5560 chromatograph (Varian, Walnut Creek, CA) monitored with a Vista 402 data system (Varian) and equipped with an autosampler (Varian 8085), an ultraviolet detector (Varian UV 200), a fluorescence detector (Varian Fluorichrom), and a 10-μL injection valve (Valco Instruments, Houston, TX). The ready-to-use C18 reversed-phase column, 4 × 125 mm, with 10-μm-diameter packing (E. Merck, Darmstadt, F.R.G.) had a 4 × 25 mm guard column (Merck). The mobile phase was potassium phosphate buffer (15 mmol/L, pH 6.45), the flow rate 0.8 mL/min.

We diluted 100 μL of urine in 1 mL of mobile phase and injected 10 μL. Neopterin was detected by its native fluoros-
ence (353 nm excitation and 438 nm emission), and creatinine by its absorption at 235 nm. Neopterin kit (control, external standard, and buffer) was purchased from Varian, Orsay, France. Intra- and interassay CVs were <5%.

Statistical Analysis

To appreciate daily changes, we plotted the data (mean ± SEM) as a function of time, each time point corresponding to the middle of the 4-h collections. The 48-h pattern reported here is the mean of the data from the three consecutive weeks. We tested the significance of peak—trough differences by Student's t-test. The effects of time, day, and week of collection were assessed by three-way analysis of variance.

Results

All the results are plotted in Figure 1. The pattern of creatinine (mmol/4 h) showed a trough at 0230 hours, a diurnal increase with a maximum at 1830 hours (2P <0.01), and a 33% total variability.

The pattern for neopterin as such (nmol/4 h) showed a peak at 0630 hours, significantly different from the other time-points (2P <0.05), and a 47% total variability. The analysis of variance validated a significant effect of the time of day (P <0.00025) and a lack of effect of day and week.

Urinary neopterin expressed as a ratio to creatinine (μmol/mol) showed high values at 0230 and 0630 hours, which correspond to urines collected from 0030 to 0830 hours. The differences between peak (167 ± 9 μmol/mol) and trough (111 ± 7 μmol/mol) values (mean ± SEM of three experiments) were statistically significant (2P <0.001, Student's t-test) and accounted for 51% of the variability. Three-way analysis of variance also validated the highly significant effect of time of day of collection (P <0.00001) and the lack of effect of day or week of collection.

Discussion

Our data clearly show a circadian rhythm of the urinary neopterin/creatinine ratio with high values at 0230 and 0630 hours that correspond to voidings collected from 0030 to 0830 hours. Evidently, neopterin is mainly excreted at night.

Because neopterin assay results are currently expressed as the ratio to creatinine, it was necessary to show that the observed variations were not related to parallel variations in creatinine. Our data show that neopterin, as such, had a prominent rhythm with a single peak at 0630 hours, i.e., during the 0430–0830 collection; the circadian rhythm of creatinine was therefore not responsible for the circadian rhythm of the ratio, although the high value of the ratio at 0230 hours was mainly ascribed to the concurrent decrease in creatinine excretion. Neopterin is considered to reflect the activation of T-lymphocytes. We note in this connection the circadian rhythm in circulating T-lymphocytes subtypes (OKT3, OKT4, NK cells) in healthy subjects with a peak at 0430 hours (7), thus preceding that of urinary neopterin. Last, data on the night peak of urinary neopterin allow us to explain the lack of variation described in another study dealing only with daytime voidings (9).

In conclusion, our study clearly demonstrates a circadian rhythm of urinary neopterin of large amplitude (51%), with a peak at 0630 hours. These results must be therefore kept in mind when neopterin is assayed. As neopterin assay is usually performed on urinary fractions and not on the 24-h collection, it is imperative to use, in a given patient, the same time-qualified voidings (preferably the first morning one), to avoid any misinterpretation in the follow-up of the patients caused by the circadian rhythm of the marker. This is of special importance when day-to-day variations are a critical element of clinical survey, e.g., monitoring of graft rejection. Besides, the large circadian variation of urinary neopterin may explain the differences observed when serum and urine assays are compared (10).

References


