Interference of Ofloxacin with Determination of Urinary Porphyrins

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The second-generation quinolone ofloxacin interferes with the screening test of porphyrins. We observed a 20-fold increase in the porphyrin concentration measured in urine of an ofloxacin-treated patient, compared with drug-free normal urine. Two other fluorinated 4-quinolones tested, norfloxacin and ciprofloxacin, had a less marked effect (a twofold increase), whereas the first-generation quinolone, nalidixic acid, did not affect the measured porphyrin concentration at all. The interference is probably due to the overlap in the emission fluorescence spectra of ofloxacin and urinary porphyrins at ~600 nm. To avoid a false-positive diagnosis of porphyria, we suggest using HPLC to separate ofloxacin (10-min retention time) from urinary porphyrins (which only start to elute at 12 min). Nonetheless, given a threefold increase in urinary porphyrins observed in the urine of an ofloxacin-treated patient, we also discuss a possible interference of the drug with the metabolism of porphyrins.

Indexing Terms: quinolones/analytical error/chromatography, reversed-phase/fluorometry/porphyria

Various drugs affect the excretion and (or) determination of urinary porphyrins and may lead to a false-positive diagnosis of porphyria.

Lim et al. previously showed that vitamin-enriched yeast tablets contain various porphyrins, and that their consumption results in an excretion pattern of porphyrins that resembles that of variegate porphyria (1). The drug dipyridamole was shown to elute with uroporphyrin and coproporphyrin in one HPLC method, and with coproporphyrin and protoporphyrin in another, leading to erroneous results (2).

This work shows the interference of ofloxacin and a few other related quinolones, all of which induce photosensitivity (3–6), in the determination of urinary porphyrins. We describe how to obtain reliable results and thus to avoid a false-positive diagnosis of porphyria cutanea tarda.

Materials and Methods

Materials

Ofloxacin tablets, 200 mg (Tarivid; Hoechst AG, Frankfurt, Germany); norfloxacin tablets, 500 mg (Apirol; Teva, Petah Tiqva, Israel); ciprofloxacin tablets, 500 mg (Ciproxin; Bayer, Leverkusen, Germany); and nalidixic acid, 500 mg (Urigram; Trima, Maabarot, Israel), were purchased from the hospital pharmacy.

We obtained a porphyrin acids marker kit from Porphyrin Products, Logan, UT, and acetonitrile and methanol (HPLC grade) from Merck, Darmstadt, Germany. All other reagents were of the highest purity available.

Screening Test

The urine was diluted 10-fold in 0.5 mol/L H2SO4, as suggested by N. G. Abraham (New York Medical College, Valhalla, NY, personal communication), and the fluorescence was determined with a Shimadzu RF-540 spectrofluorophotometer equipped with an R-928-03 photomultiplier, at 404 nm excitation and 595 nm emission wavelengths (Shimadzu, Kyoto, Japan). Fluorescence spectra were obtained by using 404 nm as the fixed excitation wavelength and 550–700 nm as the range of emission wavelengths.

Quantification of Porphyrins by HPLC

Apparatus. The HPLC apparatus consisted of an HP 1090L solvent-delivery system (Hewlett-Packard, Avondale, PA), equipped with a Rheodyne (Cotati, CA) 7010 injector and a 100-μL external loop. A C18 reversed-phase column [100 × 4 mm (i.d.), HP Hypersil octadecylsilane, 5 μm particles] was used. Fluorometric determination was performed by a programmable fluorescence detector (HP 1046A) at 404 nm excitation and 615 nm emission wavelengths. Calculations were carried out by an HP-3393 computing integrator.

Urine preparation. The pH of the urine was adjusted to 0.5 with a few drops of 4 mol/L HCl. After centrifugation for 10 min at 3000g, 100 μL was injected directly onto the column.

Drugs. The various tablets were suspended in water, sonicated, and diluted to the desired concentrations, either in 1 mol/L HCl for HPLC separation or in 0.5 mol/L H2SO4 for fluorometric scanning.

Standards. The porphyrin acids marker kit, containing 10 nmol each of 8-, 7-, 6-, 5-, 4-, and 2-carboxylic porphyrins, was dissolved in 2 mL of 3 mol/L HCl (5 μmol/L). After 20-fold dilution in 1 mol/L HCl, this solution was centrifuged and 100 μL injected as described above.

Separation procedure. The method of Lim and Peters (7) was modified for our system (8). The mobile phases were (A) 100 mL/L acetonitrile in methanol and (B) 100 mL/L acetonitrile in 1 mol/L ammonium acetate, pH 5.1. The elution plan was: linear gradient from 100% B to 35% B for 30 min, followed by 12 min of linear gradient from 35% B to 10% B, 5 min of isocratic elution, and an additional 5 min for returning to 100% B, at a flow rate of 1 mL/min.
Results

The urine of a patient suspected of having porphyria, who was being treated with ofloxacin (200 mg twice daily) for a urinary tract infection, was screened for porphyrins. The value obtained was 20-fold higher than the value measured in normal urine. The fact that the fluorescence observed was green instead of the typical red of porphyrins led us to investigate whether the high value obtained in the screening test was a false-positive caused by reaction with ofloxacin.

HPLC analysis of porphyrins was performed in urines collected during treatment with ofloxacin (Fig. 1A) and 1 week after completion of the therapy (Fig. 1B). As shown in Fig. 1 and calculated in Table 1, a threefold increase in total urinary porphyrins was observed during the treatment with ofloxacin, not enough to explain the 20-fold increase obtained in the screening test. The cause of this difference is probably the unidentified fluorescent component (10-min retention time) not normally found in the urinary porphyrin profile, which was detected only in the urine tested during treatment with ofloxacin. The peak that eluted at 10 min, as well as the peak for uroporphyrin (12-min retention time), were collected and scanned fluorometrically. As demonstrated in Fig. 2, the unknown compound eluting at 10 min is not a characteristic porphyrin molecule. To study the possibility that this peak consisted of ofloxacin, we suspended and sonicated a tablet of ofloxacin (200 mg) in water, adjusted this to pH 0.5 with 4 mol/L HCl, centrifuged, and injected an aliquot into the HPLC (final concentration, 0.09 mmol/L). A peak with the retention time of 10 min was observed when injected with a porphyrin mix (Fig. 1C). The fluorescence spectrum of this peak was identical to that of the peak collected 10 min after injecting a urine from the ofloxacin-treated patient (not shown).

Two other quinolones tested, ciprofloxacin (0.25 mmol/L) and norfloxacin (0.25 mmol/L), were eluted at similar retention times: 9.5 and 10.2 min, respectively. Although their concentrations exceeded that of ofloxacin by about threefold, the peak areas obtained were only 3% and 2%, respectively, of the peak area of ofloxacin (not shown). Nalidixic acid (0.35 mmol/L) could not be detected (not shown). We conclude that the interference of ciprofloxacin with porphyrin determination is less pronounced than that of ofloxacin; indeed, in urine of a patient treated with ciprofloxacin, only a twofold increase in the apparent porphyrin concentrations (screening test) was observed (not shown).

The fluorescence spectra of the four quinolones mentioned and uroporphyrin are shown in Fig. 3. The overlap in the spectra of ofloxacin and uroporphyrin near 600 nm is well demonstrated and is probably the cause of interference of ofloxacin with the screening test.

Discussion

The second-generation quinolone, ofloxacin, interfered with the screening test for porphyrins, leading to a 20-fold increase in the value obtained. Two other fluorinated 4-quinolones, norfloxacin and ciprofloxacin, had

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Table 1. Urinary porphyrins (nmol/day) during and after treatment with ofloxacin.

<table>
<thead>
<tr>
<th></th>
<th>Uroporphyrin</th>
<th>Coproporphyrin I</th>
<th>Coproporphyrin III</th>
<th>Total porphyrins</th>
</tr>
</thead>
<tbody>
<tr>
<td>During therapy</td>
<td>142</td>
<td>550</td>
<td>841</td>
<td>1533</td>
</tr>
<tr>
<td>After therapy</td>
<td>130</td>
<td>154</td>
<td>280</td>
<td>564</td>
</tr>
<tr>
<td>Normal range</td>
<td>&lt;72</td>
<td>&lt;92</td>
<td>&lt;275</td>
<td>&lt;439</td>
</tr>
</tbody>
</table>

* Urine collected during 24 h 3 days after starting treatment with ofloxacin (200 mg twice daily).
* b Urine collected during 24 h 1 week after completion of therapy.
* c Determined at our institution.
a less marked effect (a twofold increase) on porphyrin determination, whereas the first-generation quinolone, nalidixic acid, did not affect the determination.

The interference of ofloxacin with the determination of porphyrins is probably due to the overlap in the fluorescence emission spectra of these two molecules at ~600 nm. Although the intensity of the fluorescence of porphyrins at 404/595 nm was ~50-fold more than that of ofloxacin, the concentration of the latter in urine is >1000-fold more than that of the former, a fact that might explain the interference of ofloxacin with the determination of porphyrins. Whereas normal urine contains up to 0.44 μmol/L porphyrins, the concentration of ofloxacin (200 mg twice daily) may exceed 0.55–0.85 mmol/L (200–300 mg/L) (9), owing to the high metabolic stability of the drug (10). Most of an oral dose of ofloxacin (70%–98%) is recovered unchanged in the urine; a small amount (2%–4%) is excreted unchanged in the feces (11). Thus, one may calculate that, even when the concentration of ofloxacin in urine is low, it may still cause a slight but false increase in the porphyrin measurement. Therefore, this drug should be excluded before collecting urine for porphyrin analysis.

Whenever the screening test is strongly positive, HPLC should be used for further identification of the fluorescent material. Several HPLC methods have been described for the analysis of porphyrins in urine. Every method is subject to interference by drugs. In many cases the amount of administered and excreted drugs is much larger than the amount of the physiological compound sought (1, 2). Use of a rapid HPLC method may yield a poor separation and confuse the “strange” peak with the peaks of normal metabolites. To separate the fluorescent material in the patient’s urine, we used Lim’s 45-min HPLC method (7, 8). The huge peak of ofloxacin was observed 10 min after injection of the patient’s urine, whereas the first porphyrin did not elute until 12 min. Thus, using a shorter HPLC separation procedure (<20 min) could very well result in coelution of the two peaks and lead to a conclusion that uroporphyrin is markedly increased. This finding, together with the fact that quinolones share a common property of inducing photosensitization (3–6), may lead to a false-positive diagnosis of porphyria cutanea tarda. To avoid erroneous results, we also suggest that, besides using a 45-min separation method, one should collect and fluorometrically scan any unidentified fluorescent peak. The spectrum obtained should be compared with a typical spectrum of porphyrin. The spectrum of the 10-min peak for ofloxacin does not resemble the typical spectrum of a porphyrin molecule.

After unmasking the effect of ofloxacin, we observed that urinary excretion of porphyrins was still significantly higher during treatment with the drug than that obtained in the patient’s urine 1 week after completion of therapy. The increase described could be a secondary phenomenon accompanying a urinary tract infection or could simply reflect bacterial porphyrins present in the urine. However, it might also indicate an unknown effect of ofloxacin on one of the enzymes of the heme biosynthetic pathway.

To the best of our knowledge, no research has been conducted concerning the effect of ofloxacin or other fluoroquinolones on porphyrin synthesis. In one case of nalidixic acid-induced pseudoporphyria, no change was observed in porphyrin analysis of blood, urine, and stool (12). A study aimed at elucidating the correlation between administration of ofloxacin and porphyrin excretion is currently being carried out. Meanwhile, ofloxacin should be prescribed with caution to porphoric patients.

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References