Cefoxitin Interferes with the “Clini-Skreen” Column Method for Urinary 17-Hydroxycorticosteroids

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Cefoxitin interferes with determination of urinary 17-hydroxycorticosteroids. The apparent concentration of hormone is increased from three- to 10-fold in samples from patients receiving cefoxitin when the Amberlite XAD-2 “Clini-Skreen” column is used. To determine the mechanism of interference, we reacted aqueous solutions of cefoxitin, cortisol, cortisone, and 11-deoxycortisol with phenylhydrazine; recorded the adsorption spectra; and determined the molar absorptivities and the equilibrium and rate constants. Also, we recorded the absorption spectra of phenylhydrazine with eight other cephalosporin antibiotics and benzylpenicillin. Cortisol, cortisone, 11-deoxycortisol, and cefoxitin react with phenylhydrazine and absorb light with superimposable spectra and absorption maxima of 410 nm. The other antibiotics react with phenylhydrazine but absorbance of the products vary, none being at 410 nm. Cortisol, cortisone, and 11-deoxycortisol react with phenylhydrazine 35-fold faster, have equilibrium constants ninefold greater, and have molar absorptivities 1.6 times that of cefoxitin. Thus, cefoxitin interferes with determination of urinary 17-hydroxycorticosteroids by forming a chromophore with the same absorbance maximum and with a molar absorptivity similar to cortisol, but much more slowly.

The Porter–Silber reaction is used to determine the concentration of 17-hydroxycorticosteroids (cortisol, cortisone, 11-deoxycortisol) and their tetrahydro metabolites in urine. Such measurements are useful in conjunction with other tests in evaluating adrenal function and reserve (1). The kidneys excrete cortisol mainly as tetrahydrocortisone glucuronide (1). In the solvent-extraction method for determination of 17-hydroxycorticosteroids, β-glucuronidase hydrolyzes the steroid conjugates to the free form, and the free steroids transfer to the dichloromethane fraction, while other, possibly interfering, compounds are removed by carbon tetrachloride (2). Reaction of the extract with phenylhydrazine in sulfuric acid forms a product with a peak at 410 nm (2). One measures the absorbance at 380, 410, and 440 nm, the first and last wavelengths being used for the Allen correction. The organic solvents are difficult and hazardous to work with, so a more recent and easier method is separation with an Amberlite XAD-2 Cliniskreen column (Brinkmann Instruments) followed by reaction with phenylhydrazine and measurement at 410 nm without an Allen correction.

Cefoxitin, a cephamycin antibiotic known to interfere with the Jaffé method for creatinine (3), interferes with the Porter–Silber solvent-extraction method for urinary 17-hydroxycorticosteroids (4). Interference by cefoxitin with the column method and the mechanism of interference have not been investigated. In this study we examined the interference with the column method to demonstrate its mechanism and evaluate the potential interference of eight cephalosporin antibiotics and benzylpenicillin.

Materials and Methods

Apparatus

We used a Model 25 spectrophotometer (Beckman Instruments, Inc., Brea, CA 92621) for absorbance measurements of isolated reactions and a Gilford spectrophotometer for absorbances made after separation with the Cliniskreen column.

Reagents

Clini-Skreen urinary 17-hydroxycorticosteroid kit, cat. no. 3506261 (Brinkmann Instruments, Inc., Westbury, NY 11590). The assay is standardized with cortisol at 5, 10, 20, and 25 μg/L.

Phenylhydrazine HCl (recrystallized, "Sigma" grade; Sigma Chemical Co., St. Louis, MO 63178).
Cortisol (hydrocortisone, Sigma).
Cortisone (Sigma).
11-Deoxycortic (Sigma).
Cefoxitin sodium, sterile (lot no. 0673H; Merck, Sharp & Dohme, West Point, PA 19046).
Cephalexin sodium for injection (Eli Lilly & Co., Indianapolis, IN 46285).
Moxalactam disodium for injection (Eli Lilly & Co.).
Cefotaxime sodium (Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876).
Cefamandole nafate for injection (Eli Lilly & Co.).
Cephalirin, sodium salt, cephalaxine monohydrate, cephaloridine (Sigma).

The phenylhydrazine was dissolved in a solution consisting of nine parts reagent-grade ethanol and 16 parts concentrated sulfuric acid. We dissolved all other compounds in ethanol.

Experimental Design

Clinical studies: We determined the concentration of 17-hydroxycorticosteroids in 24-h urine specimens with the Clini-Skreen method for patients receiving cefoxitin, with a repeat analysis two days after termination of therapy.

Cortisone study: To evaluate the response of the column method to cortisone, we added cortisone (in alcohol) to a normal urine to give final concentrations of 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L. We determined the value for 17-hydroxycorticosteroids, analyzing the results by linear regression.

Cefoxitin interference study: Using the Clini-Skreen method, we determined the apparent concentration of 17-hydroxycorticosteroids in urine, from a pool, containing various concentrations (0, 0.5, 1.0, 1.5, 2.0 g/L) of cefoxitin. We calculated the sensitivity of the Clini-Skreen method to cefoxitin by regressing the apparent concentration of 17-hydroxycorticosteroids vs the concentration of cefoxitin.

Mechanistic studies: We conducted kinetic experiments with 7 mmol of phenylhydrazine per liter, 5 or 10 mg of cortisol per liter, and 50 or 100 mg of cefoxitin per liter. We mixed 100 mg of the remaining antibiotics per liter with 10 mmol/L phenylhydrazine and recorded the spectra 24 h later.

To determine rate constants, we plotted \(-\ln[A_{eq} - A_i]\) vs time, where \(A_{eq}\) is the absorbance at equilibrium and \(A_i\) is the absorbance at time \(t\). The slope of this plot is equal to the pseudo-first-order rate constant, \(k_1\), for the reaction.

To determine molar absorbivities and equilibrium constants, we plotted \([\text{reactant}]_0[A_{eq}]\) vs \(1[\text{phenylhydrazine}]_0\) according to the Benesi–Hildebrand equation, where \([\text{reactant}]_0\) is the initial concentration of either cortisol, cortisone, 11-deoxycortisol, or cefoxitin, and \([\text{phenylhydrazine}]_0\) is the initial concentration of phenylhydrazine (5). The molar absorbivity, \(\epsilon\), is equal to the reciprocal of the \(y\)-intercept and the equilibrium constant, \(K\), is equal to the \(y\)-intercept divided by the slope. We analyzed the data by linear regression and calculated the mean and standard deviation of multiple separate determinations (n).

The fraction of compound (cortisol, cefoxitin, etc.) converted to product is given by \(F = K[\text{phenylhydrazine}]_0/(1 + K[\text{phenylhydrazine}]_0)\). In addition, we subtracted the Allen correction (mean of absorbance at 380 and 440 nm) from the total absorbance at 410 nm for each compound.

Results

Clinical studies. The apparent urinary excretion of 17-hydroxycorticosteroids by patients receiving intravenous cefoxitin was increased, the values ranging from three- to ninefold the baseline value (Table 1). The interference was so great with the urine samples of patient no. 1 that they had to be diluted 10-fold to remain within the limits of linearity of the standard curve.

Cefoxitin interference study. The concentration of cefoxitin present in urine and the apparent concentration of 17-hydroxycorticosteroids are linearly related (Figure 1). The sensitivity of the Clini-Skreen method was 9 mmol of pseudo-17-hydroxycorticosteroids per mole of cefoxitin.

Cortisone study. The response of the column method to cortisone is 5.2 ±0.6 (slope ± standard error) times greater than that of cefoxitin.

Mechanistic studies. Phenylhydrazine formed products with cortisol, cortisone, 11-deoxycortisol, and cefoxitin that have superimposable spectra (Figure 2), the absorbance maximum for each product being 410 nm. The plots of \(\ln(A_{eq} + A_i)\) vs time were linear, which indicates that each reaction is pseudo-first-order with respect to the compounds reacting.

| Table 1. Interference by Cefoxitin with Determination of 17-Hydroxycorticosteroids (17-OHCS) in Urine from Three Patients |
|-----------------|---------|----------|------------------|
| Patient | Sex | 17-OHCS with cefoxitin* | 17-OHCS without cefoxitin* | Cefoxitin, g/day |
| 1 | M | 59.0 | 6.5 | 6 |
| 1 | M | 58.0 | 7.4 | 6 |
| 2 | F | 17.7 | 6.3 | 4 |
| 3 | M | 23.8 | 7.8 | 6 |

*In units of mg of 17-OHCS in urine per 24 h. Reference intervals: female, 3.0–6.0; male, 3.0–10.0.

Fig. 1. Effect of cefoxitin on determination of 17-hydroxycorticosteroids by the Clini-Skreen column method

There is a linear relationship between the amount of cefoxitin added and the value for 17-hydroxycorticosteroids: the sensitivity is 9 mmol of pseudo-17-hydroxycorticosteroids for every mole of cefoxitin.
Fig. 2. The spectra of the reaction products of phenylhydrazine with cefoxitin, cortisol, cortisone, and 11-deoxycortisol. The spectra are superimposable.

with phenylhydrazine. The molar absorptivity for the cefoxitin-phenylhydrazine product is 62% that of the cortisol; that of the cortisone-phenylhydrazine product is 22% greater than that of cortisol-phenylhydrazine (Table 2). The molar absorptivities of the products of the reaction between phenylhydrazine and cortisol or 11-deoxycortisol are the same.

The equilibrium constants for the reaction of phenylhydrazine with cortisol, cortisone, and 11-deoxycortisol are similar, but that of cefoxitin is one-fourth that of cortisol (Table 2). Given an initial concentration of 7 mmol of phenylhydrazine per liter, the fraction of hormone converted to product exceeds 95%; that for cefoxitin is 85%.

The rates of reaction of phenylhydrazine with cortisol and 11-deoxycortisol are similar, but the rate for cortisone is significantly ($P < 0.01$) slower (Table 2). The rate of reaction of cefoxitin with phenylhydrazine is much slower than that for any of the 17-hydroxycorticosteroids, being 3.4% that of cortisol (Table 2).

Phenylhydrazine reacts with benzylpenicillin, moxalactam, cepahirin, cefazolin, cefotaxime, cephamandol, and cephalothin, but none of the products have an absorption maximum at 410 nm (Table 3). The potential interference of each of these antibiotics is best understood when the absorbance of product is evaluated at the same concentration of drug, say 500 mg/L, and compared with that of cefoxitin at equilibrium (the absorbance is combination of the molar absorptivity and equilibrium constant, Table 3). The absorbances in Table 3 were all experimentally determined, except for that of cefoxitin, which was calculated. The phenylhydrazine products of cephalaxin, cepahirin, cefazolin, cefotaxime, and cephalothin had absorbances >2.0 and could potentially cause interference, but if the Allen correction were applied, only cephalaxin and cefotaxime would continue to contribute more than 20% of their absorbances (Table 3).

**Discussion**

Cefoxitin interferes significantly with the Clini-Skreen column method for the determination of 17-hydroxycorticosteroids. The patients excreted normal amounts of 17-hydroxycorticosteroids, but when they were receiving cefoxitin the determined values were high and thus would be interpreted as abnormal (Table 1). The interference is highly reproducible, as seen with patient no. 1, whose treatment had been interrupted. The doses given the patients were typical, and even higher doses, up to 12 g per day, are recommended in severe infections (6). Thus 17-hydroxycorticosteroids cannot

| Table 2. Kinetic and Equilibrium Data (50 °C) |
|-----------------|-----------------|-----------------|-----------------|
| $\epsilon$, 410 nm, L/mol cm$^2$ | Equil. constant, L/mol | Rate constant x 100, min$^{-1}$ | Half-life |
| Cefoxitin | 13 500 (931)* | 831 (148) | 0.215 (0.14) | 5.4 h |
| Cortisol | 21 800 (1500) | 3324 (360) | 6.24 (0.31) | 11 min |
| Cortisone | 26 700 (470) | 5120 (500) | 4.97 (0.31) | 14 min |
| 11-Deoxycortisol | 21 700 (400) | 7300 (140) | 6.87 (0.48) | 10 min |

* $n = 2$, except for cefoxitin, where $n = 3$ and cortisol where $n = 4$.

* Parentheses: standard deviations are shown in parentheses.

| Table 3. Absorbance Characteristics of Phenylhydrazine-Antibiotic Products |
|-----------------|-----------------|-----------------|
| Compound | Wavelength maxima, nm | $A_{410}$ nm |
| Benzylopenicillin | 400 | 0.649 |
| Moxalactam | 335, 365, 460 | 0.718 |
| Cephapirin | 390 | 1.976 |
| Cephalexin | 400 | 2.423 |
| Cephalexidine | 340, 450 | 1.289 |
| Cefazolin | 375 | 3.075* |
| Cefotaxime | 390 | 3.125* |
| Cefprozolin | 390 | 2.351 |
| Cephalothin | 370, 470 | 2.850* |
| Cefoxitin | 410 | 12.0 |

Measured at 24 h and 50 °C. The concentration of all antibiotics was 500 mg/L, cefoxitin absorbance was calculated.

* Diluted fivefold for measurement.
be accurately determined when cefoxitin is being administered to a patient.

The linear relationship between the amount of cefoxitin added and the increase in the determined value for 17-hydroxycorticosteroids denotes a mole-for-mole relationship between cefoxitin and interference (Figure 1). Given that 85% of cefoxitin is excreted unchanged (6), we estimated the interference to be equivalent to 6 mg of 17-hydroxycorticosteroids per gram of cefoxitin in urine.

The steroid hormones cortisol, cortisone, and 11-deoxycortisol react with phenylhydrazine to form products that have superimposable spectra. The product of cefoxitin with phenylhydrazine absorbs light with a spectrum identical to those of these steroid hormones (Figure 2). This absorbance at 410 nm by the cefoxitin-phenylhydrazine product and cefoxitin’s ability to co-elute with the 17-hydroxycorticosteroids cause the interference.

The typical concentration of cortisol is 27 μmol per liter of urine, while patients treated with cefoxitin have approximately 11 mmol of antibiotic per liter of urine. Thus, patients being treated with cefoxitin have about 400-fold more molecules of cefoxitin than of cortisol in their urine. The fraction of cortisol converted to product is 95%, of cortisone 97%, of 11-deoxycortisol 98%, and of cefoxitin 82%, as calculated on the basis of the equilibrium constants. We do not observe a huge absorbance by the cefoxitin product, because of its much slower rate of reaction compared with the 17-hydroxycorticosteroids (Table 2), cefoxitin reacting with phenylhydrazine at a rate 3.5% that for cortisol. This difference explains why cefoxitin, with a 400-fold greater concentration than cortisol, falls within the expected range of values for 17-hydroxycorticosteroids. If the rate of reaction for cefoxitin was on the same order of magnitude as cortisol, then the absorbance in the test would be too high to measure.

We estimated the relative number of molecules of cefoxitin eluted compared with cortisol by examining the theoretical absorbances and calculated that 0.136 mol of cefoxitin is eluted for every mole of cortisol. The other molecules of cefoxitin must either be eluted with the column washes or remain adsorbed to the column.

The molar absorptivity of the cortisone-phenylhydrazine product is 22% greater than that for products of phenylhydrazine with cortisol or 11-deoxycortisol. We calculated the absorbance per millimole of products to be 20.2 for cortisol, 24.5 for cortisone, and 20.8 for 11-deoxycortisol. The absorbance difference between the 11-deoxycortisol-phenylhydrazine product and that for cortisol is not statistically significant, whereas the difference between the cortisol and cortisone-phenylhydrazine products is. Because the column method is calibrated with cortisol, the regression analysis shows that for every mole of cortisone present in a patient’s urine, five moles of cortisol will be reported. Also, there is a greater analytical recovery of cortisone than cortisol with the method. This difference could be medically significant for individual patients.

Other penicillin or cepha antibiotics may interfere with the Porter-Silber assay. We found that none of the nine antibiotics tested formed a phenylhydrazine product with an absorbance peak at 410 nm (Table 3). The products of cephalxin, cefazolin, and cephalothin with phenylhydrazine had the highest absorbances at 410 nm, but their absorbances were only a quarter that for cefoxitin. Because in some of the solvent-extraction methods the Allen correction is applied, we calculated the contribution to absorbance relative to that of cortisol. Benzylpenicillin and moxalactam (besides cefoxitin) would have the largest effects, but because their absorptivity at 410 nm is small, the interference is insignificant. The fact that the absorption maximum for the cefoxitin-phenylhydrazine product is the same as those for the 17-hydroxycorticosteroids is a matter of chance and not ascribable to structural similarities of the products.

One would like to minimize the interference of cefoxitin by modifying the variables in the reaction or estimating the concentration of cefoxitin. The former is not practical, but the presence of cefoxitin could be detected by measuring the absorbance of the reaction mixture at three approximately equal time intervals (30, 60, and 90 min at 60°C). The reaction of phenylhydrazine with the 17-hydroxycorticosteroids will show little increase in absorbance after the first measurement, whereas the reaction with cefoxitin will show considerable increase in absorbance between the first and third. Thus, one could detect the interference and prevent the reporting of an erroneous result.

References