Oxaprozin Cross-Reactivity in Three Commercial Immunoassays for Benzodiazepines in Urine

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Abstract

Immunoassay methods are commonly used to screen for drugs of abuse and some prescription drug classes as part of drug-testing programs in clinical and forensic toxicology. Oxaprozin (Daypro) is a new nonsteroidal anti-inflammatory drug that is widely prescribed in North America and has been reported to cross-react for benzodiazepines in several different immunoassay methods. The first objective of this study was to characterize the immunoreactivity of oxaprozin standards over a wide concentration range when analyzed by the EMIT dau, Abbott FPIA, and BMC CEDIA urine benzodiazepine assays. The second objective was to measure the immunoreactivity of urine specimens obtained from 12 subjects after receiving a single oral dose (1200 mg) of oxaprozin. Urine oxaprozin standards were prepared in drug-free urine at seven concentrations ranging from 500 to 100,000 ng/mL. The standards gave presumptive positive benzodiazepine results between 5000 and 10,000 ng/mL (EMIT dau) and approximately 10,000 ng/mL (FPIA, CEDIA). With a 200-ng/mL cutoff for benzodiazepines in these assays, all 36 urine specimens collected from the 12 subjects gave positive results by EMIT and CEDIA, and 35 of 36 urine specimens were positive by FPIA. It was concluded that presumptive positive benzodiazepine results by these immunoassays may be due to the presence of oxaprozin or oxaprozin metabolites. It is recommended that all positive immunoassay screening tests for benzodiazepines be confirmed by another technique based upon a different principle of analysis.

Introduction

For approximately 20 years, diagnostic manufacturers have marketed immunoassay products for the screening of benzodiazepines and metabolites in serum and urine. The development of specific antibodies for a drug class such as the benzodiazepines is more difficult than for other drug classes because of the availability of approximately 40 different benzodiazepines around the world. Currently in Canada, 14 different benzodiazepines are marketed. Most benzodiazepines undergo significant metabolism, and cross-reactivity to several of these metabolites was reported previously (1–3).

Oxaprozin (4,5-diphenyl-2-oxazolepropanoic acid, Daypro) is a new nonsteroidal anti-inflammatory drug that was first patented by Wyeth in 1969 and licensed to G.D. Searle & Company in 1988. Daypro was approved by the U.S. Food and Drug Administration in 1993 for once-daily treatment of rheumatoid arthritis and osteoarthritis and was approved for the Canadian market by Health Canada effective 1 January 1997.

Oxaprozin (Figure 1) is well absorbed after oral doses and is extensively metabolized in the liver, including glucuronic acid conjugation, before excretion in the urine. Peak-plasma concen-
trations are found within 3–6-h postdose in subjects following a single 1200-mg dose. The mean steady-state plasma concentration of oxaprozin after multiple doses (600 mg twice/day) was 200 mg/L (4,5).

Since the introduction of Daypro to the American market in 1993, it has become a widely prescribed nonsteroidal anti-inflammatory agent. In 1995 and 1996, Daypro was number 76 and 67, respectively, on the list of the 200 most frequently prescribed drugs (new prescriptions) dispensed in community pharmacies in the U.S. (6,7).

In 1995, two laboratories reported false-positive immunoassay results in urine benzodiazepine assays that were due to the presence of oxaprozin and/or metabolites. The first report by Camara (8) described presumptive positive immunoassay results for benzodiazepines in four patients and three volunteers who had taken oxaprozin. All urine collections in that study were collected at random times after single or multiple doses of oxaprozin. This report indicated that certain urine specimens were considered presumptive positive for benzodiazepines and/or metabolites included in their test menu (and at different cutoff values). None of the ten urine specimens that screened positive were confirmed for benzodiazepines and/or metabolites by gas chromatography–mass spectrometry (GC–MS).

Another study (9) in 1995, reported on three urine specimens collected in an athletic drug-abuse program that screened positive in the EMIT II urine benzodiazepine assay, and none of the three specimens confirmed positive by GC–MS. The confirmation method used acid hydrolysis to convert benzodiazepines to benzophenones before analysis. The GC–MS assay was able to detect six different benzodiazepines/metabolites at unspecified cutoff concentrations.

The package insert for the TDxFLx analyzer (10) states that oxaprozin will give a result above the sensitivity of the FPIA benzodiazepine assay at an oxaprozin concentration of 1,000,000 ng/mL. The package insert (11) for the Behring EMIT dau assay (November 1995) does not state that oxaprozin will cross-react in their benzodiazepine assay even at very high concentrations. The BMC CEDIA DAU package insert (12) for their benzodiazepine assay (November 1995) stated that oxaprozin had 1.9% cross-reactivity in their benzodiazepine assay at an oxaprozin concentration of 10,000 mg/mL.

The 1994 edition of The Physicians Desk Reference (PDR) had a section on Daypro that stated “False positive urine screening tests for benzodiazepines have been reported in patients taking Daypro. This is due to cross-reactivity. Confirmatory testing is recommended when such screening test results are positive”.

Because of the widespread use of immunoassays to screen for benzodiazepines in urine and the concern about oxaprozin cross-reactivity in several urine benzodiazepine assays, the following study was performed. The cross-reactivity of oxaprozin standards prepared in drug-free urine was assessed over a wide concentration range, and urine specimens were analyzed up to 72-h postdose in subjects following consumption of a single oral dose (1200 mg) of oxaprozin.

**Methods**

Oxaprozin pure standard was obtained from Searle Pharmaceutical (Skokie, IL). Standard solutions of oxaprozin were prepared in drug-free urine at the following concentrations: 500, 1000, 2000, 5000, 10,000, 50,000, and 100,000 ng/mL. Oxaprozin standards were analyzed as unknown samples by the Abbott FPIA urine benzodiazepine assay (TDxFLx analyzer), EMIT dau urine benzodiazepine assay (Syva ETS analyzer), and by the BMC CEDIA DAU benzodiazepine assay (Ciba Corning Express 550 analyzer). Oxaprozin standards were analyzed by each immunoassay on five separate days. The Express 550 analyzer was programmed to give a positive result for any specimen with an absorbance (at 570 nm) greater than the CEDIA nitrazepam 200-ng/mL cutoff calibrator. The ETS analyzer was programmed to give a positive result for any specimen with an absorbance at 340 nm.

![Figure 3. Immunoreactivity of oxaprozin standards in the CEDIA DAU benzodiazepine assay.](image)

**Figure 3.** Immunoreactivity of oxaprozin standards in the CEDIA DAU benzodiazepine assay. (◆ cutoff calibrator, ■ low control, ● oxaprozin, ▼ intermediate control, ▲ high control.)

![Figure 4. Immunoreactivity of oxaprozin standards in the EMIT dau benzodiazepine assay.](image)

**Figure 4.** Immunoreactivity of oxaprozin standards in the EMIT dau benzodiazepine assay. (□ cutoff calibrator, ◇ oxaprozin, ▲ high control, ◇ low control.)
greater than the EMIT dau 200-ng/mL cutoff calibrator. The FPIA assay was set to give positive results for any specimen with a net polarization reading lower than the 200-ng/mL cutoff calibrator. Assay calibration and frequency of analyzing control samples was performed as specified by each manufacturer.

Urine specimens were obtained from 12 subjects (6 female, 6 male) enrolled in a pharmacokinetic study comparing oxaprozin with piroxicam by Searle (13). The subjects ranged in age from 18 to 43 years. Urine specimens were collected at the following times: baseline (when available), 0–24, 24–48, and 48–72 h following a single oral dose of oxaprozin (1200 mg). All urine specimens were kept frozen until analyzed in duplicate by all three immunoassay systems.

Results

Cross-reactivity of oxaprozin standards in the Abbott FPIA assay is found in Figure 2. The 10,000 ng/mL standard had a net polarization value slightly higher than the 200-ng/mL nordiazepam calibrator (negative result). An oxaprozin concentration of approximately 12,000 ng/mL had an FPIA response equal to the 200-ng/mL cutoff (1.7% cross-reactivity). The higher oxaprozin standards (50,000 and 100,000 ng/mL) always gave positive results by FPIA. Cross-reactivity of oxaprozin in the BMC CEDIA DAU benzodiazepine assay is found in Figure 3. The 10,000-ng/mL oxaprozin standard had absorbance values slightly lower than the 200-ng/mL nitrazepam cutoff calibrator. An oxaprozin concentration of approximately 11,000 ng/mL had a CEDIA response equal to the 200-ng/mL cutoff (1.6% cross-reactivity). The higher oxaprozin standards (50,000 and 100,000 ng/mL) gave positive results in each run. Cross-reactivity in the Behring EMIT dau assay is found in Figure 4. The 10,000-ng/mL standard had absorbance values slightly higher than the 200-ng/mL oxazepam calibrator. An oxaprozin concentration of approximately 8000 ng/mL had an EMIT response equal to the 200-ng/mL cutoff (2.5% cross-reactivity). The higher oxaprozin standards (50,000 and 100,000 ng/mL) always gave a presumptive positive result for benzodiazepines.

The FPIA net polarization values compared to urine collection times for the 12 subjects are found in Figure 5. In the 0–24- and 24–48-h urine collections, all 36 urine specimens were “presumptive” positive for benzodiazepines. In the 48–72-h collection, one subject’s urine specimen had a net polarization value greater than the value for the 200-ng/mL nordiazepam calibrator. The remainder of the urine specimens (35) were “presumptive” positive for benzodiazepines. Mean net polarization values of urine specimens compared with urine collection times for the 12 subjects are found in Figure 6. Baseline urine specimens were available for 4 of the 12 subjects, and FPIA benzodiazepine assays were negative for each of these specimens. The CEDIA DAU absorbance values compared with urine collection times for the 12
subjects are found in Figure 7. All 36 specimens collected at 0–24, 24–48, and 48–72 h were positive for benzodiazepines. Mean CEDIA DAU absorbance values compared with the urine collection times for the 12 subjects are found in Figure 8. Baseline urine specimens were available from 2 of the 12 subjects and the CEDIA DAU benzodiazepine assays were negative in each specimen. The EMIT dau absorbance values compared with urine collection times for the 12 subjects are found in Figure 9. All urine specimens collected at 0–24, 24–48, and 48–72 h were presumptive positive for benzodiazepines. Mean EMIT dau absorbance values compared with the urine collection times for the 12 subjects are found in Figure 10. Baseline urine specimens were available for 4 of the 12 subjects, and all 4 specimens were negative for benzodiazepines.

Discussion

Qualitative and semiquantitative immunoassay screening tests for drugs/metabolites in urine are now the most widely used screening tests for drugs of abuse in clinical and forensic laboratories today. Despite the availability of specific monoclonal antibodies, test systems which are designed to detect many different drugs/metabolites within a single drug class remain susceptible to interfering substances such as other drugs as shown recently (14) for the EMIT II opiate assay. It has been demonstrated in this study that "presumptive" positive results for benzodiazepines with the Abbott FPIA, Behring EMIT dau, and the BMC CEDIA DAU assays may be due to non-benzodiazepine drugs such as oxaprozin. It was demonstrated that the percent cross-reactivity of oxaprozin is very low (1.6–2.5%) compared with benzodiazepine/metabolite cross-reactivities as shown in the manufacturer’s package inserts (10–12). The standard daily dose of oxaprozin (1200 mg), however, is much higher than any recommended daily benzodiazepine dose. Low-dose benzodiazepines such as Halcion® (triazolam) are prescribed in doses less than 0.5 mg per day. Even older benzodiazepines such as diazepam and chlor Diazepoxide have maximum recommended daily doses of 40 mg per day. The baseline urine specimens from the 12 subjects in the study were all negative for benzodiazepines when tested by Searle Pharmaceutical. Negative screening results were also found in all baseline urine specimens tested in this laboratory. It is essential that all presumptive positive immunoassay results for benzodiazepines in urine be confirmed by another technique based upon a different principle of analysis such as high-performance liquid chromatography or GC–MS.

References


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