Oxaprozin Interference with Urinary Benzodiazepine Immunoassays and Noninterference with Receptor Assay

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Introduction

Immunoassay methods are widely used to screen for urinary benzodiazepines despite several reports of false-positive and false-negative results (1). False-negative results are produced for certain benzodiazepines that have no or low cross-reactivity with the immunoassay used. False-positive results were produced by four commercial immunoassays (Behring EMIT d.a.u., Behring EMIT II, Abbott FPIA, Microgenics/BMC CEDIA, and Biosite Triage) in subjects who had taken oxaprozin (Daypro®) (2–4). Oxaprozin, a nonsteroidal anti-inflammatory drug, is widely prescribed in North America. Oxaprozin and/or its metabolite in urine may directly interfere with these immunoassays because of their cross-reactivities with oxaprozin (4).

Benzodiazepines in body fluids can also be determined by a receptor assay that is based on competitive binding using a benzodiazepine-specific receptor in mammalian brains (5,6). Previous studies of ours and others show that the diagnostic performance of the receptor assay was equal or superior to that of some commercial immunoassays for the urinary screening (7–9). We investigated whether the receptor assay would be interfered with by oxaprozin intake.

Materials and Methods

Urine specimens were collected from five subjects following a single oral dose of oxaprozin (1200 mg) at the times 0–24, 24–48, and 48–72 h in Phase 1. In Phase 2, the same subjects received the same dose each day at time 0, 24, and 48 h with timed urine specimens being collected in the same manner. They received no benzodiazepines or any other drugs during the study. All specimens were kept frozen until analysis.

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The specimens were analyzed by three immunoassay methods (EMIT d.a.u., FPIA, and CEDIA). The cutoff value for oxazepam, nordiazepam, and nitrazepam was 200 ng/mL, as indicated by the manufacturers. Part of the results in Phase 1 (a single dose) was already reported (4).

The receptor assay was performed by the reported method (7). Urine (200 mL) was extracted with ether (2.0 mL), and two portions of a half-volume of the extract were evaporated to dryness in a separate tube for duplicate assay. The stock receptor suspension prepared from rabbit brain homogenate and [3H]flunitrazepam solution (NEN Research Products) were

Figure 1. Standard curve of oxazepam obtained by the receptor assay. Radioactivity bound to the receptor (duplicate assay results and the mean) is plotted against oxazepam concentration in urine. The bound radioactivity for the specimens was 6780–8100 dpm (shown in bar, average 7228 dpm, n = 30) and judged to be negative. The cross-reactivity relative to oxazepam in the receptor assay was 1.1 for nitrazepam, 2.5 for diazepam, 2.5 for nordiazepam, 12.5 for lorazepam, and 50 for triazolam. These cross-reactivities are correlated with their pharmacological potency.
added to the tube. The mixture was incubated at room temperature for 1 h, the assay mixture was filtered through a glassfiber membrane, and the radioactivity on the membrane was measured. A standard curve was constructed by analyzing urine calibrators of oxazepam 0, 100, 200, and 1000 ng/mL that were included in the EMIT d.a.u. kit (Figure 1). The detection limit of the receptor assay was equivalent to 75 ng/mL oxazepam, which is equal to 30 ng/mL nordiazepam and 70 ng/mL nitrazepam in the receptor-binding activity (7,8).

Results and Discussion

All three immunoassays produced positive results for all specimens (Phases 1 and 2) collected after 0 h and until 72 h. The urine collected during 0–24 h after the single dose showed the highest value (4). The urine specimens collected just before the dosage (at time 0) were all negative.

The receptor assay produced negative results, giving values below the detection limit of 75 ng/mL oxazepam for all the specimens, even for the specimens collected after total dosage of 3 × 1200 mg oxaprozin in Phase 2 (Figure 1).

We concluded that the receptor assay was not interfered with by a single or multiple (up to triple) once-daily dose of oxaprozin. The chemical structure of the immunoassay-interfering substance may be similar to that of benzodiazepines; however, this benzodiazepine-like substance will have no pharmacological activities such as antianxiety, sedation, and muscle relaxant because it does not bind to the receptor.

References


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