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Buflomedil Interference with the Monoclonal EMIT d. a. u. Amphetamine/ Methamphetamine Immunoassay

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Summary: The interference of buflomedil with the monoclonal and polyclonal EMIT d. a. u. amphetamine immunoassays was investigated. Urine samples collected from 20 patients taking 600 mg of buflomedil daily gave false positive results with the monoclonal EMIT d. a. u. assay, as did urine specimens collected 2 hours after the first oral dose of buflomedil. Conversely, no false positive results occurred with the polyclonal EMIT d. a. u. amphetamine assay. Urine samples with buflomedil added at concentrations greater than 100 mg/l gave false positive results with the monoclonal immunoassay. Buflomedil concentrations found in the patient urines (56–400 mg/l) failed to correlate to EMIT assay responses: this result suggests that one or more buflomedil metabolites, besides the unchanged drug, probably interfere in the monoclonal EMIT d. a. u. assay.

Introduction

A urine specimen submitted to toxicological screening tested positive in the EMIT d. a. u.™ (Syva Company) monoclonal amphetamine/methamphetamine assay and negative in the polyclonal amphetamine assay.

The confirmation analyses performed by high performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS) found no evidence for amphetamines, amphetamine-like compounds, or other substances structurally unrelated to amphetamines known to produce positive results with the Syva Company monoclonal immunoassay test for amphetamines (1–3). Bio-Rad REMEDi™ (4, 5) analysis suggested the presence of buflomedil, and GC-MS and HPLC analyses confirmed the result. Buflomedil hydrochloride, 4-(1-pyrrolidyl)-1-(2,4,6-trimethoxy-phenyl)-1-butanone hydrochloride, is a vasodilating agent used in the treatment of cerebrovascular and peripheral arterial diseases. Recommended therapeutic doses for adults are usually 300–600 mg orally, or 50–200 mg intravenously, per day.

The present study was undertaken to verify the interference of buflomedil with the monoclonal and polyclonal EMIT d. a. u. amphetamine assays.

Materials and Methods

Study protocol

Urine specimens were collected from 20 patients (7 males and 13 females, ranging from 34 to 91 years of age) receiving orally 600 mg of buflomedil hydrochloride daily. Urine samples from 2 other patients were collected after 0, 2, 4, 6 hours from the first oral dose of 600 mg of buflomedil hydrochloride.

Buflomedil was added to drug-free urine specimens to determine the response of monoclonal and polyclonal EMIT d. a. u. assays for amphetamines.

Some patients routinely received drugs other than buflomedil. These drugs were identified and checked for possible interference by performing amphetamines with immunoassays on drug-free urine samples spiked with 100 mg/l of each drug.

Analytical methods

All samples were screened with the Bio-Rad REMEDi Drug Profiling System and by in-house chromatographic methods (HPLC with diode array detector and GC with nitrogen/phosphorus detectors), in order to check for the possible presence of compounds known to produce a false positive response with amphetamine EMIT d. a. u. assays. The monoclonal and polyclonal EMIT d. a. u. Syva tests were performed in a Random 120 analyzer as recommended by the manufacturer: in particular, the cutoff concentration of the monoclonal assay was 1 mg/l of *D*-methamphetamine and the cutoff concentration of the polyclonal assay was 0.3 mg/l of *D*-amphetamine. HPLC quantitation for buflomedil was performed on all urine samples using a method including a liquid-liquid extraction at pH 9 with diethylether/methylene chloride (70 + 30, by vol.) containing clothiapine as internal standard. Chromatographic conditions were: mobile phase, 2.88 g/l sodium lauryl sulphate in water/acetonitrile/glacial acetic acid (45 + 55 + 0.5, by vol.); column, Lichrosorb Select B Merck; diode array detector wavelength, 275 nm.

Results and Discussion

All urine specimens of patients treated with buflomedil yielded a positive response with the monoclonal EMIT d. a. u. amphetamine assay. Also the urines collected at 2, 4, 6 hours after the first oral dose of buflomedil (2 patients) were positive; the urine samples collected at time 0 were negative. Conversely, all urine samples ana-

lyzed using the polyclonal EMIT d. a. u. amphetamine assay were negative. The toxicological screening performed on all urines excluded the presence of drugs known to produce false positive with the monoclonal immunoenzymatic test. The drugs, other than buflomedil, tested negative when added to drug-free urine specimens at 100 mg/l. The urine samples were found to contain 56–400 mg/l of buflomedil when analyzed by the HPLC method.

Figure 1 presents the responses of the monoclonal EMIT d. a. u. amphetamine assay plotted against the buflomedil concentrations measured in the urines from treated patients.

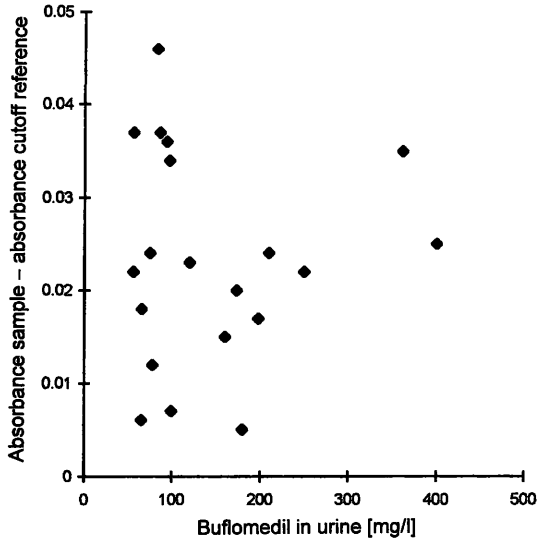

Fig. 1 Scattergram of the results of monoclonal EMIT d. a. u. amphetamine immunoassay performed on urine samples from buflomedil treated patients (n = 20).

The response of the immunoassay was linearly related to the concentration of parent drug added to the sample over the range of 50–400 mg/l (r = 0.9841). Buflomedil-added samples containing 100 mg/l gave a reaction rate of the cutoff or very close to it, while the real samples containing buflomedil concentrations significantly lower than 100 mg/l gave rates greater than the cutoff. These results indicated that one or more buflomedil metabolites can also be interfering compounds. In fact, both on the REMEDi and in-house HPLC chromatograms, the presence of peaks presumably due to metabolites of buflomedil was always observed. Since no reference standards were available for the metabolites of buflomedil, their identification was based on the similarity of their UV spectra to that of the parent drug. The data are consistent with the buflomedil excretion pattern (6–8).

In conclusion, therapeutic doses of buflomedil can cause false positive results in the EMIT monoclonal amphetamine test. In our laboratory we have observed three cases of acute poisoning due to buflomedil overdosage, where the urine screenings for amphetamines performed with monoclonal EMIT d. a. u. Syva gave a positive result. Such a laboratory finding can generate confusion for a correct diagnosis, as some important clinical features due to buflomedil poisoning (agitation, convulsions, tachycardia, respiratory insufficiency) are common to amphetamine overdosage (6, 9–11).

The confirmation of a positive amphetamine screening assay is absolutely necessary, nevertheless buflomedil interference can be easily revealed in urine by chromatographic methods.

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