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TECHNICAL NOTE

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Cross-reactivity of the CEDIA buprenorphine assay with opiates: an Austrian phenomenon?

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Abstract When testing the Microgenics CEDIA assay for immunological buprenorphine analysis, cross-reactivity between the buprenorphine reagents and opiates was observed at concentrations higher than 120 mg/l morphine, 320 mg/l methadone, 30 mg/l codeine, 60 mg/l dihydrocodeine and 520 mg/l morphine-3-glucuronide. The cross-reactivity with morphine has the greatest impact on routine screening as opiate maintenance therapy in Austria is also performed with slow-release oral morphine. The use of a second cutoff value of 30 μg/l for urine samples that are (immunologically) positive for opiates is therefore suggested, compared to the cutoff value of 5 μg/l proposed by the manufacturer.

Keywords Buprenorphine · CEDIA · Opiates · Cross-reactivity · Opiate maintenance therapy

Introduction

One possibility to treat addiction to opiates, especially heroin, is maintenance treatment with substitution agents. The substance of first choice is still methadone [1, 2], but even heroin has been tested for maintenance therapy in different countries [3–6]. Nevertheless, it is buprenorphine that has gained more and more importance in addiction treatment because the correlation between dose and therapeutic effects is not linear, indicating a ceiling on the effects in patients due to its opiate agonistic–antagonistic characteristics [7–9]. Buprenorphine is therefore a relatively safe substance, and its effectiveness in maintenance therapy has been proved in many studies [10–17]. It has been used in Austria as a substitution drug since 1999. In 1998, slow-release oral morphine was also established for substitution [18, 19], which gives Austria an exceptional position in opiate addiction treatment compared to, for example, Germany.

An essential part of the maintenance therapy is the regularly performed toxicological screening of urine samples of the patients [18], thus proving the opiate addiction and subsequently the taking of the substitution drug. An important point is also the detection of illicit consumption of these substances, not only in the substituted patients, but also in forensic cases. An opiate differentiation by GC-MS is performed routinely in all cases of positive immunological results for opiates in urine samples at our institute.

In 2004, the Microgenics Corporation (Passau, Germany) launched a new CEDIA assay for the immunological detection of buprenorphine, which had been tested by the manufacturer for possible cross-reactivity, for example, with methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), codeine, dihydrocodeine, heroin or morphine up to a spiked urine concentration of 100 mg/l (instruction for CEDIA buprenorphine assay, Microgenics).

During validation processes of the CEDIA buprenorphine assay at our institute, some unwanted reactivity was observed, as an unrealistically high number of urine samples of patients in maintenance therapy were tested positive for buprenorphine. Therefore, a possible cross-reactivity with the commonly used opiates was checked, and a retrospective data analysis of the tested urine samples was performed.

Materials and methods

Urine samples were immunologically screened on a Hitachi 902B according to the manufacturer's instructions using CEDIA reagents from Microgenics.

Blank urine samples were spiked separately with morphine, codeine, dihydrocodeine, methadone and morphine-3-glucuronide at concentrations of 25, 50, 100, 150, 200, 500, 1000 and 1500 mg/l and tested six times on two different days for buprenorphine with the CEDIA kit. The

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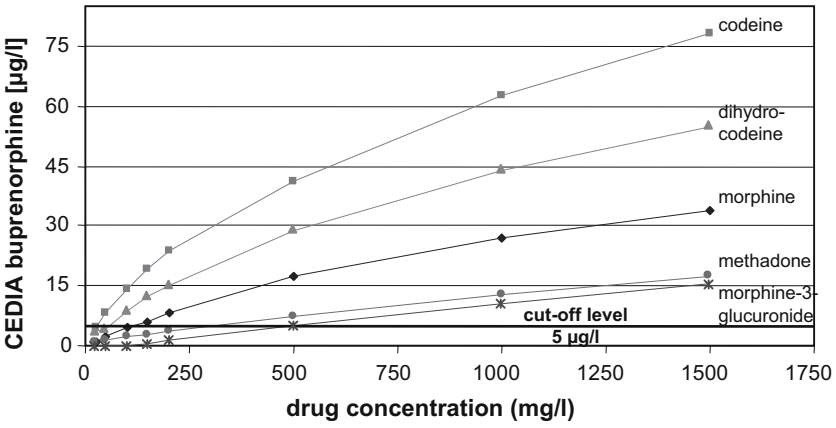

Fig. 1 If the recommended cut-off level of 5 µg/l is used, the CEDIA buprenorphine assay gives positive results with urine concentrations higher than about 30 mg/l for codeine, 60 mg/l for dihydrocodeine, 120 mg/l for morphine, 320 mg/l for methadone and 520 mg/l for morphine-3-glucuronide

cutoff value was defined according to the manufacturer's recommendation as 5 µg/l. A negative control was tested after each measurement series.

For semiquantitative analysis of the urine samples for non-conjugated morphine, methadone, codeine, dihydrocodeine and 6-monoacetylmorphine, 2 ml of urine with nalorphine as internal standard was extracted on SPE-ed matrix (Applied Separations, Vienna) filled Extrelut 3 columns (Merck, Vienna). After elution with ethyl acetate, derivatisation with pentafluoropropionic anhydride (Fluka, Vienna) and reconstitution in acetone, GC-MS analysis was performed (HP 5890 Series gas chromatography system, HP 5970 mass selective detector, Hewlett Packard, Vienna) using a DB-5MS column (20 m×0.25 mm i.d.×0.25 µm film thickness, J&W Scientific, Agilent Technologies, Vienna); carrier gas: helium at 1 ml/min, injection temperature 270°C, injection volume 1 µl; oven: 110°C for 1 min, 40°C/min to 225°C, 10°C/min to 230°C, 40°C/min to 300°C; MS: electron impact ionisation, selected ion mode (SIM); limit of detection (LOD): 100 µg/l.

For the retrospective toxicological analyses, the results of 600 urine samples of maintenance therapy patients that were analysed with the buprenorphine CEDIA kit were evaluated. Urine samples of buprenorphine-substituted patients had been excluded. The remaining samples were divided into two groups: samples containing methadone (or EDDP) and samples containing morphine. If a urine sample contained both drugs, it was assigned to the morphine group. The immunological buprenorphine results were subsequently analysed for both groups separately. The results were statistically checked with WinSTAT 2003.1 for MS Excel.

Results

The urine samples spiked with methadone were immunologically tested positive for methadone; the samples spiked with morphine, codeine, dihydrocodeine or morphine-3-glucuronide were positive for opiates. Blank urine samples were tested negative.

CEDIA testing for buprenorphine partly produced positive measurement results with each spiked substance, depending on their urine concentration. The correlation coefficient for linear regression for the whole concentration range constituted between 0.966 for codeine and 0.995 for methadone. The mean values of the six measurement results for each drug are depicted in Fig. 1. According to these curves, concentration thresholds can be calculated from where false-positive buprenorphine results begin if a cutoff value of 5 µg/l is taken. These thresholds were about 520 mg/l for morphine-3-glucuronide, 320 mg/l for methadone, 120 mg/l for morphine, 60 mg/l for dihydrocodeine and about 30 mg/l for codeine.

For the retrospective evaluation of urine samples from maintenance therapy patients, 600 samples were included, 244 samples containing morphine and 356 samples containing methadone. Of the methadone and morphine samples, eight (2.25%) and 103 (42.2%) were immunologically tested positive for buprenorphine, respectively. This difference

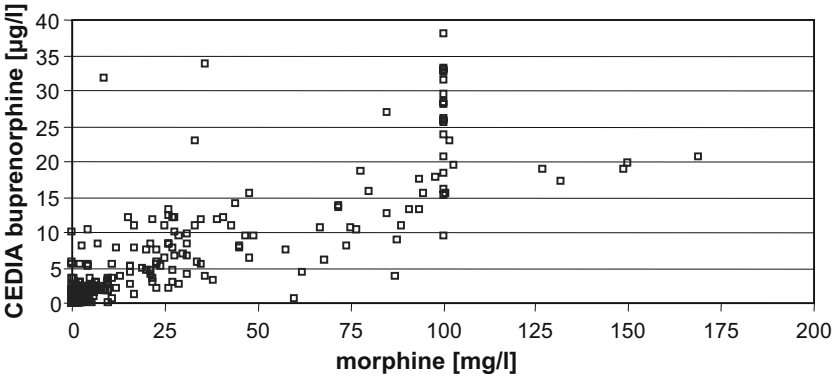

Fig. 2 CEDIA buprenorphine assay results for patient urine samples containing morphine. A correlation between morphine concentration and buprenorphine results can be observed. For a better view, buprenorphine results until 40 µg/l are depicted, thus truncating two samples containing 100 and 119 mg/l morphine, resulting in buprenorphine values of 62 and 81 µg/l, respectively

between these two groups regarding the positive buprenorphine measurement results was statistically highly significant (Mann–Whitney *U*-test, $p<0.0001$). Also, in these samples, a certain correlation ($R^2=0.561$) between urine morphine concentrations and buprenorphine results could be observed (Fig. 2). No sample contained codeine without morphine. Six of the methadone samples contained additionally dihydrocodeine, two being immunologically positive for buprenorphine.

Discussion

When performing the newly introduced CEDIA buprenorphine assay on urine samples from maintenance therapy patients, many samples from morphine-substituted patients showed a positive buprenorphine result. This was suspicious as the partial agonist/antagonist buprenorphine is known to block the effects of morphine and even to induce withdrawal symptoms [8, 9], so morphine-substituted patients are not expected to take buprenorphine additionally. Retrospective analysis then showed that over 40% of the morphine positive urine samples were tested positive for buprenorphine (Fig. 2), but only about 2% of the methadone positive urine samples. Only the latter is a value that can be explained adequately with an additional consumption of buprenorphine in single cases or with false-positive immunological results, which would be the case in about 1% of tests according to the manufacturer.

The spiked morphine concentrations between 25 and 1500 mg/l were considered as realistic, as in the patient samples, concentrations of non-conjugated morphine up to 175 mg/l were observed. This is only about 10% of the total morphine amount that is excreted in the urine, whereas 75% is excreted as morphine-3-glucuronide [20]. The results (Fig. 1) indicate a relevant cross-reactivity between CEDIA buprenorphine reagents and morphine at concentrations higher than 120 mg/l and with morphine-3-glucuronide concentrations above 520 mg/l. This is in good accordance with the information of Microgenics that no cross-reactivity was observed up to a morphine concentration of 100 mg/l. Such high levels do occur in Austria where morphine is used as a substitution drug, but are not expected in patients who receive morphine for pain therapy.

To a certain extent, cross-reactivity with CEDIA buprenorphine reagents also occurred with codeine, dihydrocodeine and methadone, in which the methadone concentration of 320 mg/l is too high to play a role in routine analysis. The different "cross-reactivity concentrations" for morphine and methadone can be explained with the similar molecular structures of morphine and buprenorphine, differing basically only in two side chains, whereas methadone has a quite different chemical structure. A careful testing of selectivity of the reacting antibodies in the development of immunological methods is therefore necessary [21]. "Cross-reactivity concentration" of codeine is in fact lower, but its role in routine analysis remains unclear as, in our patient samples, codeine was always combined with morphine.

Altogether, the cross-reactivity of CEDIA buprenorphine reagents with morphine has the most important impact on routine toxicological analyses. A feasible and easy way to handle the cross-reactivity can be the use of two different cutoff levels for buprenorphine. If a sample is immunologically positive for opiates, a cutoff value for buprenorphine of 30 μg/l, for example, instead of 5 μg/l seems realistic. Using this proposed cutoff value, only nine (3.7%) of the 244 morphine-containing urine samples had to be considered as buprenorphine positive. Thus, buprenorphine screening could still be performed for all urine samples.

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