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Evaluation of the first immunoassay for the semi-quantitative measurement of meprobamate in human whole blood or plasma using biochip array technology

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ABSTRACT

Background: Meprobamate is a carbamate, and the main metabolite of carisoprodol. It is used as an anxiolytic agent. Overdose of both drugs produces intoxication that is often serious and sometimes life threatening. However there was until now no immunoassay for the diagnosis of this intoxication.
Methods: A chemiluminescent immunoassay for the semi-quantitative measurement of meprobamate in human blood and plasma has recently been developed, using the Evidence Investigator system (Randox®). In this study, the immunoassay was evaluated by testing drug-free (n = 10) or spiked whole blood and plasma samples (n = 70), and authentic post mortem whole blood samples from deceased patients in which meprobamate was present (n = 38) or not (n = 10). A previously validated gas chromatography–mass spectrometry (GC–MS) method was used for confirmation and quantification. 97 psychoactive drugs including carisoprodol were analyzed for possible interference.
Results: With a cut-off at 0.5 mg/L, specificity, sensitivity and accuracy were 100%, 97.2% and 97.6%, respectively. All the untreated patients presented results under the cut-off. Meprobamate was not detected in three whole blood samples spiked with concentrations under the therapeutic range. In the authentic patients (n = 48), there were no false-negative results. A good correlation was found between the immunoassay and GC–MS (r = 0.90). Quantitative results of the immunoassay are approximately two-fold lower than GC–MS results. Only carisoprodol presented a cross-reactivity, 38 ± 6.6% at 10 mg/L, and 26 ± 4.8% at 100 mg/L.
Conclusion: The first meprobamate immunoassay has shown very good specificity, selectivity and accuracy, which allow its use in hospital clinical laboratories for rapid diagnosis of meprobamate (or carisoprodol) intoxications.

1. Introduction

Meprobamate belongs to the family of carbamates (Fig. 1), and is the main active metabolite of carisoprodol [1]. The first one is marketed as an anxiolytic agent and the second one as a centrally active muscle relaxant analgesic. These products induce depressant effects on the central nervous system (CNS) and have the potential to impair driving [2]. In the USA, they were in the 10 most frequently identified compounds in drug-impaired driving casework [3] and several case reports on the toxic potential of carisoprodol have been published [4,5]. Meprobamate has a barbiturate-like mode of action at the $GABA_A$ receptor and is able to induce the influx of chloride ions at high concentrations. In case of overdose, toxic effects include CNS depression, weakness, clonus, tachycardia, hypotension and respiratory depression. Death could occur resulting from hemodynamic disturbance and circulatory collapse, secondary to acute cardiac failure [6]. Meprobamate is involved in many suicide attempts and is implicated in 4 to 5% of the total deaths in France [7–9]. There is a good correlation between the blood concentration of meprobamate and its therapeutic or toxic effects [10]. Therapeutic plasma concentrations are in the range 10–20 mg/L, and toxic effects appear at concentrations greater than 50 mg/L. A non-severe coma is classically observed with concentrations between 60 and 120 mg/L, while deep comas require concentrations above 120 mg/L. Death occurs with plasma concentration up to 200 mg/L. Considering this relationship, the 24/7 monitoring of meprobamate blood concentration is of great interest for the diagnosis of acute intoxications or even for the management of meprobamate elimination following hemodialysis. Several methods have already been reported for the quantification of meprobamate in human plasma or whole blood. Most of these methods are based on gas chromatography coupled with either flame ionization [11] or mass spectrometry detection

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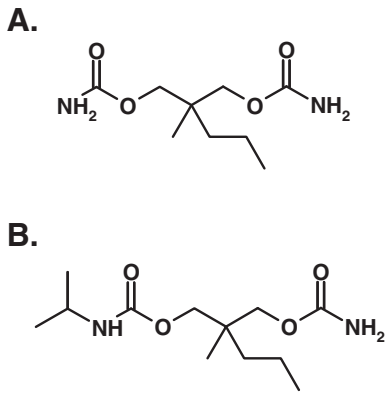

Fig. 1. Chemical structures of meprobamate (A) and carisoprodol (B).

[8,12,13], but the coupling of liquid chromatography with tandem mass spectrometry detection was also recently reported [14]. As they are time-consuming and require experienced staff, they are not appropriate for the detection of meprobamate 24/7 in emergency situations. Even if one immunoassay has been described for the rapid detection of carisoprodol in urine, there is until now no available method suitable for blood samples [15]. Since an immunoassay-based test was recently designed and developed for the semi-quantitative measurement of meprobamate in whole blood samples, the aims of the present study were to evaluate this assay and to compare the results to those obtained with gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Chemicals and reagents

A certified solution of meprobamate at 1 mg/mL in methanol was provided from LGC Standards (Illkrich, France). The internal standard mebutamate was a gift from Dr Palette (Laboratory of pharmacology, Versailles Hospital). Methanol and sodium hydroxide 32% were purchased from VWR Prolabo (Fontenay-Sous-Bois, France). HPLC grade acetonitrile and phenylboronic acid were provided by Sigma Aldrich (Saint Quentin Fallavier, France). Ultra-pure water was obtained by ultrafiltration using a Direct-Q UV3 apparatus (Millipore Corp, Molsheim, France). Drug-free human whole blood or plasma samples were supplied by the local blood bank (Etablissement Français du Sang, Le Chesnay, France).

2.2. Whole blood and plasma samples

One hundred and twenty eight samples were selected for the study. Negative samples were as follows: ten authentic post mortem whole bloods from untreated patients in which meprobamate was assayed and not detected with GC–MS, five drug-free human plasma and five drug-free whole blood from untreated patients. Thirty-eight post mortem whole blood in which meprobamate was previously found were chosen as authentic samples. Additionally, seventy whole blood and plasma samples were spiked with meprobamate in the concentration range 5–333 mg/L. At each value tested (5, 10, 25, 50, 100, 250 and 333 mg/L), five spiked whole blood and five spiked plasma samples were analyzed. All the negative and positive (either native or spiked) samples were analyzed in parallel with a validated GC–MS method and with the immunological assay.

2.3. GC–MS method

Briefly, meprobamate was hydrolyzed with sodium hydroxide 32% at 100 °C during 15 min in 250 μL of whole blood or plasma, then extracted with 500 μL of acetonitrile and derivatizated with 100 μL of phenylboronic acid 80 mM in methanol. The internal standard was mebutamate, a non-marketed carbamate. The GC–MS analysis was performed using a Focus GC (Thermoelectron, Les Ulis, France) and a DSQ II mass spectrometer (Thermoelectron) coupled with an AS 3000 Auto sampler (Thermoelectron). The chromatographic system was equipped with a 30 m × 0.25 mm i.d., Supelco PTE 5 5% phenyl 95% methylsiloxane column. The injector was set at 250 °C and used in splitless mode. The injector gas was helium N55 grade (Air Liquide, Jouy-en-Josas, France) and its pressure was maintained at 70 kPa. The GC oven temperature was programmed starting at 70 °C during 30 s and increased to 290 °C at 35 °C/min and maintained for 2 min. The transfer line temperature was set at 300 °C. The mass spectrometer was in electron impact mode (70 eV). The MS was run in selected ion monitoring (SIM) mode, using $m/z = 218$ (used as quantitative ion), 56 and 132 (used as confirmation ions) for meprobamate derivate and $m/z = 232$ and 132 for mebutamate derivate. Xcalibur software (Thermoelectron) was used for equipment control and data processing. The validation parameters of the GC–MS method were linearity in the concentration range 1–200 mg/L, and limits of detection and quantification of 0.5 and 1 mg/L, respectively. The intra- and inter-assay precisions were all < 12.3% and the intra- and inter-assay accuracies were in the 102–113% range. Our laboratory also participated to a National Quality Control program at the same period than the current study. Two levels of plasma and blood samples were obtained from the French Society of Analytical Toxicology (SFTA, Société Française de Toxicologie Analytique) and processed with GC–MS. Results were 41 and 89 mg/L for plasma samples (median values obtained from 40 laboratories were 42.4 and 90.0 mg/L respectively, corresponding to bias of −3.3% and −1.1%). For whole blood samples, the values obtained were 24 and 202 mg/L (medians for the 40 laboratories of 24.1 and 204.2 mg/L respectively, bias of −0.5% and −1.1%).

2.4. Immunoassay on the Evidence Investigator™

2.4.1. Sample preparation

Whole blood or plasma samples (n = 128) were diluted 1/200 with the sample diluent provided by the manufacturer. When the results were greater than the highest calibration point, samples were systematically diluted 1/500 and analyzed again.

2.4.2. Biochip immunoassay

A semi-automated Evidence Investigator™ analyser was used for the biochip assay investigation (Randox Laboratories Ltd., Crumlin, UK). The assay was based on competition for binding sites of a polyclonal antibody (PAS10016, Randox Laboratories Ltd., Crumlin, UK) between free meprobamate present in the sample and horseradish peroxidase-labelled conjugate (HRP9456, Randox Laboratories Ltd., Crumlin, UK). The chemiluminescent signal input was then inversely proportional to the concentration of analyte in the sample.

The antibody was immobilized and stabilised onto the biochip surface as previously described [16], and its specificity towards meprobamate and carisoprodol was documented with respective 100% and 57% cross-reactivity, according to the manufacturer. Each biochip carriers contained nine biochips suitable for the determination of meprobamate concentrations in nine individual samples. The maximum analytical capacity of the system allowed the process of 54 biochips at the same time. The experimental procedure is summarized as follows: assay diluent (155 μL), calibrator/diluted sample (25 μL) followed by working strength conjugate (120 μL) were applied to the appropriate biochip. The biochips were then incubated for 30 min at 30 °C on a thermoshaker set at 330 rpm. Following several washing steps in buffer, the signal reagent (250 μL, 1:1 luminol + peroxide, *v/v*) was added. After 2 min the biochip carrier was imaged in the semi-automated Evidence Investigator™ analyser. The instrument incorporates dedicated software to process, report and archive the data generated. With each reagent batch, a nine-point calibration curve