SHORT COMMUNICATION / KURZMITTEILUNG

Assay for Cyclo-, Seco- and Pentobarbital by Multiple Ion Detection: Kinetics after a Single Dose

By Henrik Steudel, A. Steudel and G. E. von Unruh

Medizinische Universitäts-Klinik, Bonn-Venusberg

(Received January 5/November 26, 1981)

Summary: A sensitive assay for the determination of four different barbiturates: butabarbital, cyclobarbital, pentobarbital and seco-barbital is described. The barbiturates are determined quantitatively by gas chromatography/mass spectrometry (GC/MS) as their dimethylated derivatives. The sensitivity of this simple procedure is limited to $10 \times 10^{-6}$ g/l for cyclo- and seco-barbital and $1 \times 10^{-6}$ g/l for pentobarbital in plasma. The assay has been used to measure the kinetics of pentobarbital over 6 days after a single 50 mg dose of the sodium salt, and over 3 days for one combined preparation of 25 mg cyclobarbital-calcium and 75 mg seco-barbital-sodium. The corresponding barbiturate levels in saliva were determined by the same assay.

Bestimmung von Cyclo-, Seco- und Pentobarbital durch Multiple Ion Detection: Kinetik nach Einzeldosen

Zusammenfassung: Ein empfindliches Bestimmungsverfahren für die vier verschiedenen Barbiturate Butabarbital, Cyclobarbital, Pentobarbital und Secobarbital wird beschrieben. Die Barbiturate werden quantitativ als Dimethylderivate mittels Gaschromatographie/Massenpektrometrie (GC/MS) bestimmt. Die Empfindlichkeit dieses einfachen Verfahrens ist auf $10 \times 10^{-6}$ g/l für Cyclo- und Secobartical und auf $1 \times 10^{-6}$ g/l für Pentobarbital in Plasma begrenzt. Dieses Bestimmungsverfahren wurde verwendet, um die Kinetik von Pentobarbital nach einer einzigen 50 mg Dosis des Natriumsalzes 6 Tage lang und die eines Mischpräparates aus 25 mg Cyclobarbital-Calcium und 75 mg Secobarbital-Natrium 3 Tage lang zu messen. Die entsprechenden Barbituratkonzentrationen im Speichel wurden mit denselben Methoden bestimmt.

Introduction

There have been numerous publications dealing with methods for the determination of barbiturates. GC assays of undervatilated barbiturates are not sensitive enough to measure the decline of plasma levels after one sleep-inducing dose. The factor that limits sensitivity is the activity of the GC-support material. The adsorption of barbituric acids on the column is drastically reduced by methylation. Using the convenient on-column methylation with reagents like trimethylanilinium hydroxide, sensitivity can be increased by a factor of about 100. Even then, the remaining active sites on the support limit the ultimate sensitivity (for reviews see l.c. (1–3)). We describe a GC/MS assay sensitive enough to measure barbiturate pharmacokinetics after a single dose of the lowest barbiturate formulations available on the German market. The assay has been used in 2 pharmacokinetic studies. Detailed pharmacokinetic data will be reported elsewhere.

Materials and Methods

Volunteers

Healthy volunteers participated in the kinetic studies after being informed of the purpose of the study. All gave their written informed consent. They had received no medication for at least 4 weeks prior to the administration of the barbiturates. All had normal kidney and liver function as assessed by appropriate laboratory tests.

Materials

Trimethylanilinium hydroxide was prepared as described by Broichmann-Hanssen (4) as 0.5 mol/l solution in methanol. Diethyl ether, toluene and methanol were analytical grade. (E. Merck, Darmstadt) and distilled before use. Pentobarbital capsules containing 50 mg pentobarbital as the Na-salt (Nembutal®, Abbott, Ingelheim) were obtained from our pharmacy. Capsules containing a mixture of 25 mg cyclobarbital-Ca-salt and 75 mg seco-barbital- Na-salt were obtained from Dr. K. Pfleger, Chemische Fabrik, Bamberg. The internal standards butabarbital (for pentobarbital) and pentobarbital sodium (for the measurement of the two other barbiturates) were obtained from SERVA, Feinbiochemica, Heidelberg. The mass spectrometer was an LKB 2091 GC/MS instrument with an LKB 2091-710 hard wire multiple ion detector.

GC-conditions

90 cm, 2 mm i.d. glass column, 3% OV-17 on Gas-Chrom Q, 100—120 mesh from Applied Science Laboratories Inc. State College, PA 16801. Several OV-17 coated supports from other manufacturers gave less sensitivity. Injector 260 °C, column 150 °C or 165 °C isothermal for pentobarbital or cyclo- and seco-barbital, respectively. He 30 ml/min, one injection every 2.5 or 6 min. The separator temperature was 250 °C.

MS-conditions

Ion source 250 °C, source pressure ca. $2–3 \times 10^{-7}$ Torr, 20 eV ionization energy, 50 μA trap current, accelerating voltage 3.5 kV for mass 184 and 3.29 kV for mass 196 (seco-barbital), multiplier voltage 3.4 kV.

Procedures

The studies were started early in the morning after an overnight fast. A single capsule containing either 50 mg sodium pentobarbital or 25 mg cyclobarbital calcium and 75 mg seco-barbital sodium was administered to the volunteers with 100 ml water. No food or tobacco were allowed for at least 2 hours after taking the drugs. Blood samples (6 ml) were obtained from the antecubital veins by means of an indwelling catheter before dosing and 10, 20, 30, 45 min and 1, 2, 3, 4, 6, 8, 12, 24 hours after drug intake. Thereafter blood was withdrawn by venipuncture at 36, 48, 72 hours after administration of cyclo- and seco-barbital and further at 96 and 120 hours after having received pentobarbital. The blood samples were collected in heparinized tubes, centrifuged within 2 hours and the plasma was stored at $-18 °C$ until analyzed. Unstimulated saliva was collected for ca. 10 minutes into test tubes. The saliva samples were centrifuged like the blood samples. The supernatant was used for analysis.

0340-0765X/82/0020-0267$02.00
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The extraction procedure was identical for all the barbiturates. After the administration of the combination preparation secobarbital, butabarbital (250 ng, 25 μl of a methanolic solution 10 × 10^{-3} g/l) and pentobarbital sodium (500 ng, 50 μl of a methanolic solution 10 × 10^{-3} g/l) were added to 1 ml plasma or saliva in a glass-stoppered 12 ml centrifuge tube. After addition of 1 ml of 0.1 mol/l HCl the plasma was extracted once with a mixture of 2 ml toluene and 5 ml diethyl ether by rotating for 10 minutes. After separation from the aqueous phase by centrifugation (10 minutes, 2250 g) as much as possible of the organic phase was transferred to a 10 ml glass-stoppered conical tube. The organic layer was blown down under a stream of nitrogen at room temperature to ca. 1 ml (until ether could no longer be smelled). Finally 25 μl of 0.5 mol/l trimethylanilinium hydroxide (corresponding to at least 1000 fold excess) and 25 μl deionized water were added to the toluene solution, mixed on a vortex for 15 s and centrifuged (5 minutes, 900 g). An aliquot (0.4–1 μl) of the alkaline aqueous methanolic phase containing the barbiturates and the trimethylanilinium hydroxide — was injected into the gas chromatographic column.

To test the stability of the barbiturates in the trimethylanilinium hydroxide containing extract, the following procedure was used: To 4 samples, each containing 1 ml of blank plasma, 250 ng pentobarbital and 100 ng butabarbital were added as internal standards. These samples were extracted as described above, methylated with trimethylanilinium hydroxide and measured in the GC/MS-System the same day. Afterwards the extracts were kept at -20 °C and measured again on the next and on the 7th day. From this time on the extracts were kept at room temperature and measured on the 8th and 14th day. The results are shown in the following table (tab. 1).

The mixture is stable at -20 °C for at least 1 week and decays at room temperature.

Calibration curves were constructed by analysing plasma samples spiked with different amounts of barbiturates. The concentrations of the internal standards were held constant. The calibration curves were shown to be linear in the ranges of 2–400 × 10^{-5}, 2–200 × 10^{-5} and 1–100 × 10^{-5} g/l plasma or saliva for cyclobarbital, secobarbital and pentobarbital.

The reliability of the procedure was proved by several investigations.

To show the precision of the series we took a pool of 50 ml plasma containing a concentration of 750 × 10^{-6} g/l of each of the four barbiturates. These control samples were measured together with each series of plasma taken from the volunteers. For 48 series \( \bar{x} = 754.1 ± 9.4 \times 10^{-6} \text{ g/l} \) and a coefficient of variation of 1.2% was found. The precision from day to day was shown by using calibration samples at the beginning of each measurement. The coefficient of variation was then 1.4%. Keeping the GC-column in the GC/MS system for several days or reinstalling it after the measurement of other substances had no effect on this coefficient of variation. The sensitivity was...
limited by the GC-column, and the remaining factor of 100 in mass spectrometric sensitivity could not be used. The signal to noise ratio was 0.09 to 1 at a plasma concentration of $20 \times 10^{-5}$ g/l of each barbiturate.

The reproducibility of the analysis was determined by repeated assays of plasma and saliva with known concentrations from 25 to $100 \times 10^{-5}$ g barbiturate/l plasma or saliva. The coefficients of variation ($n = 10$ for each barbiturate at $25 \rightarrow 100 \times 10^{-5}$ gA) were between 0.78 and 2.84%, thus indicating a good reliability of the measured values.

Recoveries of the barbiturates including all steps of the analytical procedure were determined by a comparison of the peak heights obtained from processed plasma and saliva samples with the peak heights of directly injected standards. Different concentrations from 0.051 —1 gA and constant concentrations of the Standards were used. The results indicated that the recoveries of the four barbiturates were in the range of 80 ± 2%.

Results and Discussion

One representative example of the plasma decay curve for each drug investigated is shown in figures 5 and 6. The pharmacokinetic parameters were calculated using the data starting with the 6 hour values. The plasma half-lives, apparent volumes of distribution and total clearances are 17.5, 19.0 and 26.5 hours, 0.4, 1.1 and 1.3 l/kg and 0.29, 0.67 and 0.58 ml/min X kg for cyclobarbital, secobarbital and pentobarbital respectively. These parameters are in agreement with the values determined after larger doses by other authors (5—7). The half-life of pentobarbital is controversial in the literature (5, 6, 8, 9); values between 15 and 60 hours have been reported. In view of the sensitivity and specificity of our method we are certain that the values measured in our 6 volunteers ranging from 17 to 40 hours are real and that the differences are due to interindividual variations.

This procedure can be used without modification for measuring the pharmacokinetics of a pulse dose of these barbiturates labelled with stable isotopes during uninterrupted treatment.

Acknowledgement

The mass spectrometer was a donation of the Dr. Robert Pfieger-Stiftung, Bamberg. This work was supported in part by the grant PTB 8203 of the Bundesministerium für Forschung und Technologie.

References


Frau Dr. Henrike Steudel
Medizinische Universitätsklinik
Sigmund-Freud-Str. 25
D-5300 Bonn 1