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Note

High-performance liquid chromatographic determination and preliminary pharmacokinetics of propofol and its metabolites in human plasma and urine

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Propofol (2,6-diisopropylphenol) in soya bean egg phospholipid emulsion is the newest intravenous anaesthetic agent to become available for clinical use [1–4]. The pharmacokinetics of the compound itself after a single bolus injection can be described by a three-compartment model with $t_{1/2}$ values of 1.8, 34 and 180 min [1]. It has been suggested that the short anaesthetic activity of propofol after a single bolus dose is the result of rapid metabolism [2]. Propofol glucuronide (40%) and the 1- and 4-glucuronide and 4-sulphate conjugates of 2,6-diisopropyl-1,4-quinol are described as the major metabolites in humans [2]. After a subanaesthetic dose of [^{14}C]propofol, 88% of the isotope is excreted in the urine as metabolites and less than 0.3% is excreted as propofol [2,3].

Adam et al. [5] have described a high-performance liquid chromatographic (HPLC) analysis of propofol in blood following coupling with Gibbs' reagent. This technique has a detection limit of 0.2 μg/ml, but fails to measure the metabolites. Kay and co-workers [1,4] analysed propofol by HPLC with fluorescence detection following extraction into cyclohexane. The limit of sensitivity of this assay is ca. 2 ng/ml. Both methods are time-consuming and do not include the

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metabolites in their analysis. Therefore a new technique for the estimation of propofol and its metabolites in human blood and urine was developed.

EXPERIMENTAL

Patients

Four unpremedicated patients undergoing superficial surgery gave their informed consent to participate in the study, which was approved by the hospital Ethics Committee.

Blood

Samples of 5 ml were taken from an arterial cannula into blood gas syringes immediately before the dose of propofol and after at predetermined times. The blood samples were centrifuged and the plasma was stored at −20°C pending analysis.

Urine

Urine was collected over predetermined time intervals and the volume and pH were measured. Urine samples were stored at −20°C pending analysis.

Chromatography

A Specta-Physics 3500B liquid chromatograph (Spectra-Physics, Eindhoven, The Netherlands) was used, equipped with a variable-wavelength spectrophotometer (Spectroflow 757, Kratos U.S.A., Rotterdam, The Netherlands). The detector was connected to a 10-mV recorder (BD 7, Kipp and Zonen, Delft, The Netherlands), and the chart speed was 1 cm/min.

A stainless-steel column (25 cm×4.6 mm I.D., Chrompack, Middelburg, The Netherlands) was packed with Spherisorb 5 ODS (Chrompack). A guard column, tap-filled with pellicular reversed-phase material, was placed between the sampling valve and the analytical column.

Propofol and its 1,4-quinone and 1,4-quinol derivatives were detected at 270 nm. The mobile phase was water–acetonitrile–methanol (40:50:10, v/v/v) with a solvent flow-rate of 2 ml/min, at a pressure of 23.30 MPa. The injection volume was 100 μl. The chromatographic analysis was carried out at room temperature.

Materials

β-Glucuronidase (*Escherichia coli*), β-glucuronidase/arylsulphatase (*Helix pomatia*) and arylsulphatase (*Aerobacter aerogenes*) were purchased from Sigma (St. Louis, MO, U.S.A.). The solvents acetonitrile and methanol were of HPLC grade from Fisons (Loughborough, U.K.). All other chemicals were of reagent grade (Merck, Darmstadt, F.R.G.). Propofol and its 1,4-quinone and 1,4-quinol derivatives were a gift from ICI (Manchester, U.K.).

Sample preparation

Plasma. To 0.2 ml of plasma in an Eppendorf reaction vessel, 0.2 ml of acetonitrile was added and mixed thoroughly on a vortex mixer. The mixture was cen-

trifuged in a Biofuge A (Heraeus Christ, F.R.G.) at 11 000 *g* for 5 min. A 100-μl volume of the clear supernatant was injected onto the column.

Urine. Urine was diluted 50 times with the mobile phase, the solution was mixed, and 100 μl were injected onto the column.

Enzymatic hydrolysis of propofol and metabolites

Plasma. To 0.2 ml of plasma in an Eppendorf reaction vessel were added 50 μl of a β-glucuronidase (*E. coli*) solution (1000 U/ml of phosphate buffer, pH 6.8). The solution was incubated at 37°C for 5 h, before 0.2 ml of acetonitrile were added. The resulting solution was mixed thoroughly, kept at 4°C, and centrifuged just before analysis. A 100-μl volume was injected onto the column.

Urine. To 10 μl of urine were added 0.2 ml of a phosphate buffer (pH 6.8) and 25 μl of the β-glucuronidase solution (1000 U/ml phosphate buffer, pH 6.8). The solution was incubated at 37°C for 5 h, before 0.2 ml of acetonitrile were added. The resulting solution was mixed thoroughly, kept at 4°C and centrifuged just before analysis. A 100-μl volume was injected onto the column.

A 10-μl volume of urine was also incubated in 0.2 ml of acetate buffer (pH 5.0) with 25 μl of a β-glucuronidase/arylsulphatase (*Helix pomatia*) solution, containing 10 000 U/ml β-glucuronidase and 403 U/ml arylsulphatase and with 100 μl of an arylsulphatase (Limpets, Sigma) solution in 50% glycerol, 0.01 *M* Tris solution (pH 7.5).

Chemical hydrolysis of plasma and urine samples

To 0.1 ml of plasma in an injection vial (2–3 ml) were added 0.4 ml of 5 *M* hydrochloric acid and 0.5 ml of methanol. The vials were tightly closed with rubber and felscap and put in a thermobath at 100°C. After 1.5 h of incubation the vials were cooled in cold water and kept at 4°C. Just before analysis the mixture was transferred to an Eppendorf reaction vessel by means of a 1-ml gas-tight syringe. The solution was centrifuged at 11 000 *g* for 5 min, and 100 μl of the clear supernatant were injected onto the column.

To 10 μl of urine were added 0.1 ml of water, 0.4 ml of 5 *M* hydrochloric acid and 0.5 ml of methanol. The solutions were treated in the same way as the plasma samples.

Testing of the analytical procedure

The accuracy, precision and linearity of the method were determined using spiked samples of human plasma and urine analysed at random. Total recovery of propofol and its metabolites was determined as the response from plasma standards relative to standards in water–acetonitrile (50:50, v/v) injected directly onto the column. Total recovery from urine was determined as the response from urine standards, relative to standards obtained by diluting standard solutions in methanol 50 times with the mobile phase, injected directly onto the column.

RESULTS

Chromatography

Propofol is well separated from its 1,4-quinol and 1,4-quinone derivatives (Fig. 1). The absorption maximum of propofol in the mobile phase is at 270 nm. At

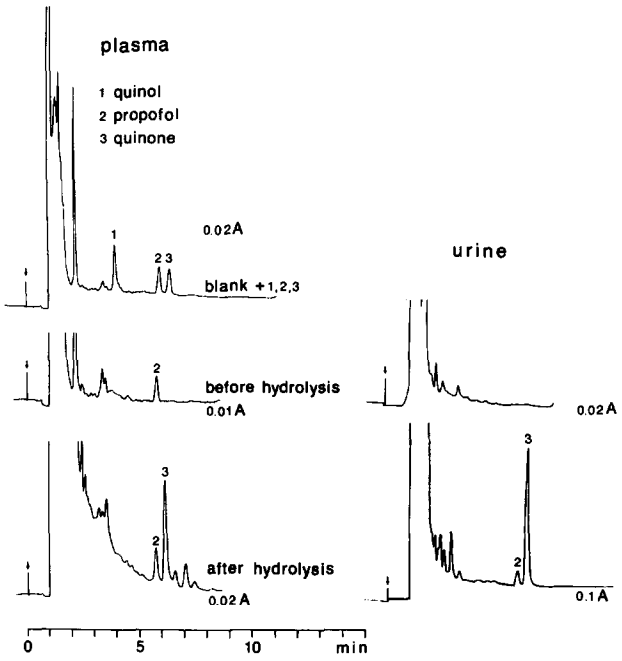

Fig. 1. HPLC profiles of propofol and its 1,4-quinol and 1,4-quinone derivatives in plasma and urine before and after hydrolysis. The concentration of propofol in plasma is 0.5 μg/ml before hydrolysis and 9.6 μg/ml after hydrolysis. The concentration of 1,4-quinone found after hydrolysis is 3.3 μg/ml. The concentration of propofol in urine after hydrolysis is 120 μg/ml and that of the 1,4-quinone 182 μg/ml.

this wavelength the absorption maximum of both the 1,4-quinol and 1,4-quinone derivatives is ca. eight times higher.

The recovery of propofol added to human plasma in the concentration range 0.1–20 μg/ml was 99.5 ± 0.5%. The recovery of the 1,4-quinone and 1,4-quinol metabolites in the concentration range 0.060–6.0 μg/ml was 100 ± 2%. The recovery for all compounds added to urine in the concentration range 10–500 μg/ml was similar to that of the plasma recovery. The precision, expressed as the relative standard deviation (% S.D., $n=6$) was 4.1% (range 3.2–5.0%) for plasma and 4.5% (range 3.3–5.5%) for urine for porpofol. The precision for the 1,4-quinol and the 1,4-quinone was 5.2% (range 4.5–6.0%) in plasma and 5.5% (range 4.6–6.2%) in urine.

Calibration curves for propofol ($r=0.9996$), its 1,4-quinone ($r=0.9990$) and 1,4-quinol ($r=0.9989$) metabolites, were linear.

Virtually all the unconjugated propofol was found in plasma; in urine it accounted for less than 0.3% of the administered dose. After deglucuronidation no increase in the propofol plasma concentration was found. Increase of the propofol concentration in plasma and urine was estimated after hydrolysis with 5 *M* hydrochloric acid. The 1,4-quinol derivative was not detected in human plasma or urine, even after hydrolysis with β-glucuronidase, arylsulphatase or 5 *M* hydrochloric acid. The 1,4-quinone metabolite of propofol appeared after hydrolysis

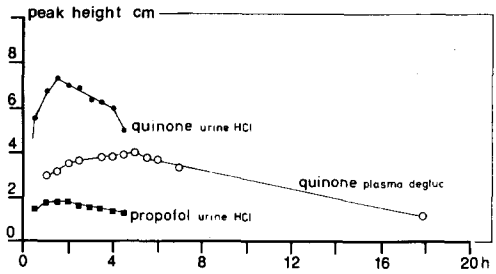

Fig. 2. Yield (peak height, cm) of some hydrolysis processes plotted against time.

with β-glucuronidase and with 5 *M* hydrochloric acid. Arylsulphatase did not hydrolyse either the propofol conjugate or the 1,4-quinone conjugate, even after a week of incubation at 37°C.

Stock solutions of propofol and its 1,4-quinone metabolite were prepared in methanol and kept at 4°C in the refrigerator. Once the 1,4-quinone solution was diluted with aqueous solutions, it became unstable. So, standard solutions in plasma and urine containing the 1,4-quinone metabolite were prepared freshly every day before analysis, and they were not incubated at 37 or 100°C. After incubation the linearity decreased, especially for the 1,4-quinone metabolite.

Fig. 2 shows optima in peak heights of free propofol and the 1,4-quinone after hydrolysis of plasma and urine samples of different patients after incubation for different periods. These optima were found 1.5 h after incubation at 100°C and 5 h after deglucuronidation at 37°C. The minimum detectable concentrations of propofol and its 1,4-quinone metabolite in plasma after hydrolysis at 100°C were 0.10 and 0.015 μg/ml, respectively, and in urine 1.0 and 0.15 μg/ml, respectively.

Fig. 3 shows the plasma concentration–time curves and renal excretion rate–time profiles of propofol with its glucuronide and of the 1,4-quinone metabolite and its conjugate in a patient after an intravenous administration of 250 mg of propofol. Propofol is extremely rapidly metabolized in its conjugate and in the 2,6-diisopropyl-1,4-quinone which was liberated after deglucuronidation and hydrolysis (sulphated). Table I summarizes the half-lives of propofol and its metabolite obtained from four patients and Table II the percentage of the dose excreted in the urine.

DISCUSSION

This HPLC method enables the measurement of the concentrations of propofol and its 1,4-quinone in human plasma and urine, and can be used for the determination in patients. Previously reported methods for the analysis of propofol do not include the analysis of the metabolites [1,2,4,5]. The enzymatic and chemical hydrolyses have to be carried out in sealed vials, owing to the volatility of propofol and the 1,4-quinone.

Propofol, as a phenolic compound, can be partly glucuronidated and sulphated [6]. The rate of enzymatic desulphatation depends highly on the molecular structure of the compound. Desulphatation can be achieved by treating the sam-

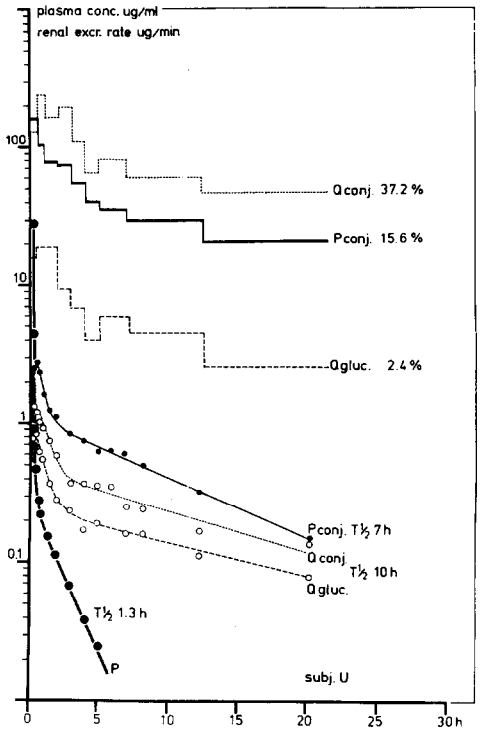

Fig. 3. Plasma concentration–time curves and renal excretion–time profiles of propofol (P), its conjugate (P_{conj}) and its metabolites 1,4-quinone conjugate (Q_{conj}) and 1,4-quinone glucuronide (Q_{gluc}) after intravenous administration of 250 mg of propofol to a patient undergoing superficial surgery.

ple with 5 *M* hydrochloric acid. It can therefore be assumed that when deglucuronidation does not occur, propofol is conjucated by sulphation or that the deglucuronidation is sterically hindered [7]. Glucuronidation of propofol may also be inhibited by the steric hindrance of the two isopropyl groups. The metabolite 1,4-quinone may be the result of chemical conversion of the 1,4-quinol, which may be glucuronidated at the 4 position and glucuronidated/sulphated at the 1 position. These two metabolites are found in plasma and urine. Table II shows that 15–33% of the parent drug is conjugated by glucuronidation/sulphation and

TABLE I

HALF-LIFE OF PROPOFOL AND ITS 1,4-QUINONE METABOLITE IN HUMANS

P = Propofol; P_{conj} = propofol conjugate; Q_{conj} = 1,4-quinone conjugate; Q_{gluc} = 1,4-quinone glucuronide.

Subject	Intravenous dose (mg)	Half-life (h)			
		P	P_{conj}	Q_{conj}	Q_{gluc}
L	432	1.3	9	9	9
K	140	1.3	20	20	20
U	250	1.3	7	10	10
D	120	1.8	13	13	13

TABLE II

PERCENTAGE OF THE DOSE EXCRETED IN THE URINE

dt = time of urine collection; P = propofol; P_{conj} = propofol conjugate; Q_{conj} = 1,4-quinone conjugate; Q_{gluc} = 1,4-quinone glucuronide.

Subject	dt (h)	P	P_{conj}	Q_{conj}	Q_{gluc}	Total
L	16	–	33.3	22.5	0.6	56.4
K	48	–	26.8	50.3	0.8	77.9
U	20	–	15.6	37.2	2.4	55.2
D	22	–	18.0	25.9	0.9	44.8

20–50% is finally oxidized to the 1,4-quinol/1,4-quinone. The 1,4-quinol is predominantly conjugated by sulphation and only 1–3% by glucuronidation. A more elaborated pharmacokinetic analysis will be presented elsewhere [8].

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