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Altered dose-to-effect of propofol due to pharmacokinetics in rats with experimental diabetes mellitus

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Abstract

Pathology related alterations in the pharmacokinetics or the pharmacodynamics of propofol could contribute to the observed large variability in the hypnotic dose. We have tested the influence of diabetes mellitus on the induction dose and the pharmacokinetics and pharmacodynamics of propofol in rats. Diabetes was induced in rats by administration of streptozotocin (60 mg kg^{-1}, i.p.) while control rats received vehicle intraperitoneally. All animals had glucose, cholesterol, triglycerides and albumin levels measured. In-vitro protein binding was determined by ultrafiltration. Rats were randomly split into set 1 (dose–concentration-effect study) with control and streptozotocin rats, and set 2 (pharmacokinetic study), with control and streptozotocin rats. Rats in the effect set received either a variable infusion of 6 mg kg^{-1} min^{-1} propofol until onset (induction dose) of the hypnotic effect (loss of the righting reflex), or a 15 mg kg^{-1} bolus to assess offset time (recovery of the righting reflex). Blood (C_{blood}) and brain (C_{brain}) propofol concentrations at onset and offset were assayed by HPLC. In the pharmacokinetic study, propofol was administered intravenously at 6 mg kg^{-1} min^{-1} for 2 min. Arterial blood samples were collected between 0.5 and 540 min and assayed for propofol. A mixed effects compartmental pharmacokinetic modelling method (NONMEM) was used to analyse the observations and variabilities. The dose necessary for onset differed between streptozotocin and controls, and so did the pharmacokinetics with two- and three-compartment descriptions, respectively. C_{blood} and C_{brain} at onset and offset were similar, possibly rejecting changes in pharmacodynamics. The total and unbound volume of distribution was significantly lower in the streptozotocin group with no differences in clearance (CL) between streptozotocin and controls, (mean (inter-animal CV%)) CL = 0.026 (17%) and 0.025 (62%) L min^{-1}, respectively. Individual Bayes Vd_{ss} (volume of distribution at steady state) were different, (mean (s.d.)) Vd_{ss} = 7.7 (2.67) and 1.11 (0.09) L, respectively. The pharmacokinetic model was validated by comparison with the data from set 1. Simulations of total and unbound C_{blood}, for both groups, at the hypnotic dose for the controls, revealed differences throughout the time course of the pharmacokinetics. The difference observed in the induction dose of propofol to streptozotocin and control rats was due to alterations in the pharmacokinetics, secondary to the pathology.

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Introduction

Propofol is used widely in anaesthesia but, besides attempts to overcome variability in the response, the identified covariate models based on e.g. body weight (Leslie & Crankshaw 1991; Chassard et al 1996), patient's age (Schnider et al 1999; Adachi et al 2001), sex (Vuyk et al 2001), rate of infusion (Stokes & Hutton 1991; Schnider et al 1998), coadministered drugs (Short & Chui 1991) or cardiac output (Adachi et al 2001), still offer inadequate predictability. Part of this problem has been attributed to the pharmacological properties of propofol. The drug shows extended compartmental volumes of distribution, elevated total clearance, high binding to plasma proteins (Altmayer et al 1995; Schuttler & Ihmsen 2000), and delay between blood and effect-site, which is typically explained via an equilibration rate (k_{e0}) (Kazama et al 1999; Wakeling et al 1999).

However, many of these studies have been performed in healthy subjects, and thus variability related to the presence of severe, or chronic illness has not been well

addressed. Diabetes mellitus is known to be associated with altered response of CNS acting drugs, such as morphine (Simon & Dewey 1981; Simon et al 1981; Courteix et al 1998), ethanol (Ohsawa & Kamei 1997) and diazepam (Ramanathan et al 1998), but the causes of such alteration have not been elucidated. It has been reported that with diabetes mellitus drug pharmacokinetics can be affected by changes in gastrointestinal absorption, an increase in the glomerular filtration rate, altered activity of metabolizing enzymes and also changes in plasma protein binding (Xiaotao & Hall 1995; Emami et al 1998). Possible changes in the pharmacodynamic properties of drugs, associated with diabetes mellitus, are even less explored (Gwilt et al 1991; Gilbert et al 1998).

Changes in the in-vitro binding of propofol to plasma protein, in patients with diabetes types 1 and 2, have been related to disease dependent alterations in the biochemical parameters (de la Fuente et al 2002). Two predictive models were established to estimate the unbound fraction of propofol based either on lipoprotein serum concentrations, or triglycerides and cholesterol. However, the importance of binding changes in the pharmacokinetics or the response of propofol under diabetes mellitus has not been determined.

In this study, we have explored the possible alterations in the induction dose of propofol and in the pharmacokinetics and pharmacodynamics in an experimental diabetes rat model. Nonlinear mixed effects modelling was used for optimum resolution of inter-animal and intergroup variabilities in compartmental pharmacokinetics, using total and unbound drug concentrations.

Materials and Methods

Chemicals and reagents

Propofol (Diprivan 1%) was obtained from AstraZeneca (Madrid, Spain). Streptozotocin was obtained from Sigma Chemical Co. (Barcelona, Spain). Chemicals for HPLC analyses were of at least HPLC grade. All other reagents and solvents were of analytical grade obtained from commercial sources.

Animals and surgical procedures

The study protocol was approved by the Committee on Animal Experimentation of the University of the Basque Country.

Adult male Sprague Dawley rats (n = 60) (250–300g) were used. The rats were maintained under standard laboratory conditions on a 12-h light/dark cycle with light from 0800 to 2000 h, in a temperature (21–22°C) and humidity (70%) controlled room, and were acclimated. The day before the experiment, a polyethylene catheter (i.d. 0.3 mm, 10-cm length; Vygon), filled with heparinized saline (50 IU heparin/mL physiological saline solution), was placed into the right carotid artery for blood sampling and another polyethylene catheter was placed and fixed into the right jugular vein to perform drug administration. Rats were fasted overnight before the experiment but water was freely available. Animals were randomly distributed into control or streptozotocin sets.

Induction of diabetes mellitus

Experimental diabetes mellitus was induced by a single intraperitoneal injection of $60\,mg\,kg^{-1}$ streptozotocin (Trancede et al 1983). Controls received vehicle intraperitoneally. The experimental diabetes mellitus model used in this study has been widely employed to evaluate the possible alterations in the pharmacokinetics and pharmacodynamics of several drugs (Danhof et al 1985; Tanaka et al 1993; Emami et al 1998). To confirm the disease state, plasma glucose concentrations were measured four weeks after injection of streptozotocin (the day before administration of propofol). Rats were considered diabetic and included in the study if their plasma glucose was greater than $250\,mg\,dL^{-1}$. Plasma glucose, fasting cholesterol, fasting triglycerides and fasting albumin concentrations were determined using standard analytical methods.

Experimental protocol

Dose–concentration-effect study (effect study)
The total dose for onset of loss of the righting reflex (induction dose), defined as the ability of the animal to move the extremities or the head upon application of pressure to the tail with forceps (Gustafsson et al 1996; Danhof & Levy 1998), was determined in control (C_{on}; n = 10) and streptozotocin (STZ_{on}; n = 10) rats. Propofol was administered intravenously at $6\,mg\,kg^{-1}\,min^{-1}$ until the endpoint. The rats were then decapitated using a small animal guillotine and samples of brain and blood taken immediately.

The time to effect offset (sleep time), defined as return of the righting reflex or the ability of the animal to reposition itself on four legs, was assessed in control (C_{off} ; n = 7) and streptozotocin (STZ_{off}; n = 5) rats post $15\,mg\,kg^{-1}$ propofol bolus. Immediately at offset, rats were decapitated with the small animal guillotine, and blood and brain samples obtained. During the time that they remained dormant, the rats were kept over an electric blanket at constant temperature to avoid hypothermia.

Pharmacokinetics study
A control group (C_{pk}; n = 8) and a streptozotocin group (STZ_{pk}; n = 10) were used to study the pharmacokinetics. All animals received the corresponding dose of propofol as an infusion of $6\,mg\,kg^{-1}\,min^{-1}$ for 2 min. Arterial blood samples were collected at intervals from 0.5 to 540 min post-dose (n = 11) and placed in heparinized tubes. Different volumes of blood were drawn (50–100 μL) to provide sufficient drug for detection. Blood was replaced with an equal volume of saline. The maximum total amount of blood withdrawn was 2 mL per rat.

Propofol assays in blood, plasma and brain

Propofol levels in blood (C_{blood}), plasma (C_p) and brain (C_{brain}), from respective samples, were determined by high-performance liquid chromatography (HPLC) with

fluorescence detection, according to a previously described method (Chan & So 1990) with minor modifications (Zamacona et al 1998).

Propofol was extracted from blood and plasma samples by the two-step extraction procedure. Briefly, samples (100 μL, except for the 540-min time point with 200 μL) were mixed with 10 μL thymol, as internal standard (8 $\mu g\,mL^{-1}$ in methanol) and 1 mL NaH_2PO_4. The contents of the vials were extracted with 5 mL cyclohexane (2.5 mL in each step) by shaking mechanically for 15 min at 12 rev min^{-1} followed by centrifugation for 5 min at 4500 rev min^{-1} at 4°C. The supernatant organic layer was transferred carefully to a glass tube containing 50 μL ammonium tetramethylhydroxide (TMAH) solution (25% TMAH in methanol with 2-propanol (7.5% v/v)) and was evaporated to dryness. The residue was reconstituted in the mobile phase and 50 μL was injected into the HPLC system for analysis. Detection was performed by fluorescence (excitation and emission wavelengths were 276 and 310 nm, respectively). The mobile phase, a mixture of acetonitrile, distilled water and glacial acetic acid (60:40:1, v/v/v), was eluted at 2.5 mL min^{-1} with a Nova-Pack C_{18} (100 mm × 8 mm) reverse phase column. The total run time for each sample was approximately 8 min. The coefficient of variation was $< 10\%$ in the concentration ranges encountered in the study and the lowest limit of quantitation in blood and plasma samples was 0.020 $\mu g\,mL^{-1}$ for the 100-μL samples.

The propofol assay in brain samples (0.2 g brain homogenized in 500 μL NaH_2PO_4) was performed according to the same procedure described for blood and plasma. The coefficient of variation was $< 10\%$ and the lowest limit of quantitation in brain samples was 0.3 $\mu g\,g^{-1}$. The extraction recoveries from blood, plasma and brain homogenate had (mean, range) 83.6% (75.8–87.8%), 81.1% (78.1–84.2%), 55.3% (55.1–56.5%), respectively, across a range of concentrations and the corresponding standard curves.

Calibration curves for blood, plasma, and brain were constructed on each experiment day.

In-vitro protein binding study

In-vitro plasma propofol binding was investigated in a separate set of 10 rats, assigned randomly either to streptozotocin ($n = 5$) or control ($n = 5$) groups. Plasma (900 μL), mixed with 10 $\mu g\,mL^{-1}$ propofol (previously established within the order of therapeutic concentrations), was transferred to Amicon Micropartition Units (MPS-1). The devices contain a membrane filter of controlled porosity with a 10 000-Da molecular weight cut-off that retains plasma protein and allows free drug in solution to pass through. The MPS-1 was centrifuged at 3000 rev min^{-1} for 8 min at 37°C. This procedure is considered as a reliable and relatively straightforward system to use for separating the free propofol in the blood sample from the protein-bound compound. The free propofol concentration obtained as ultrafiltrate (C_u) was measured by direct injection in the HPLC system described above. The lower limit of quantitation of propofol was 5 ng mL^{-1}.

The unbound fraction percent in plasma (fu_p) was determined as:

$$fu_p = (C_u/C_p) \times 100 \quad (1)$$

The unbound fraction in blood (fu) was estimated based on fu_p scaled by the relation between C_{blood} and C_p calculated from the effect study rats ($BRP = \Sigma C_{blood}/\Sigma C_p$, where ΣC is the sum from set 1 of all C_{blood} and all C_p, respectively).

$$fu = fu_p \times BRP \quad (2)$$

Data analysis

Noncompartmental analysis was initially performed for control and streptozotocin rat groups, for qualitative analysis of the two groups. The parameters examined were systemic clearance (CL), mean residence time (MRT) and steady state volume of distribution (Vd_{ss}) (WinNonlin 1.5, Pharsight Inc., Mountain View, Palo Alto, CA).

Population (mixed effects) pharmacokinetic modelling was used to obtain the statistical distribution for compartmental pharmacokinetic parameters for control and streptozotocin rat groups (for both groups as a single population and for each group apart). The method resolves the inter-animal variability in the parameters as well as the residual errors between observations and models (intra-animal), facilitating robust comparisons, and also model validation and simulation. Two- and three-compartment models were applied with a Nonlinear Mixed Effects Modeling (population) approach (NONMEM, Regents of the University of California San Francisco, CA) using the first-order conditional estimation (FOCE) method. The parameter distributions obtained from NONMEM were then used as Bayesian priors to obtain the individual rat pharmacokinetic parameters (empirical Bayes parameter estimates).

The model for the j^{th} concentration measurement in the i^{th} individual was

$$C_{ij} = f(\theta_i, t_{ij}) + \varepsilon_{ij} \quad (3)$$

where $f(\theta_i, t_{ij})$ was the fitted compartmental pharmacokinetic model, θ_i was the structural model parameters for the i^{th} rat, t_{ij} represented the time of the j^{th} concentration measurement in the i^{th} rat, and ε_{ij} was the residual or unexplained difference between the model prediction and the observed response. ε_{ij} was modelled as normally, independently distributed with mean $E[\varepsilon_{ij}] = 0$ and variance $Var[\varepsilon_{ij}] = \sigma^2\, f(\theta_i, t_{ij})^2$, so σ was a constant coefficient of variation (CV = s.d./mean).

The pharmacokinetics parameters for the i^{th} individual (θ_i) were modelled as:

$$\theta_i = \bar{\theta} \times e^{\eta_{\theta i}} \quad (4)$$

where $\bar{\theta}$ represented the typical (similar to the mean) population value for each parameter and $\eta_{\theta i}$ was the corresponding random effect (inter-animal parameter variability) arising from the lognormal distribution $LN(\bar{\theta}, \omega_\theta)$. This model resulted in estimates of inter-animal variance (ω^2) that were squared coefficients of variation for the parameters.

Structural pharmacokinetics model selection was based on several criteria: the NONMEM objective function (minus twice the logarithm of the maximum likelihood, −2LLD); standard errors for the parameter estimates; the magnitude of the intra-individual variability; and the visual inspection of plots of weighted residuals vs the predicted variable.

Validation and simulation

The observed C_{blood} in the C_{on} and STZ_{on} rats (from the effect study) were used for validation of the pharmacokinetic model (derived in the pharmacokinetic study). Monte Carlo (MC) simulations (10 000) were performed for the expected C_{blood} time course and 95% confidence interval ($CI_{95\%}$ = mean ± 1.96 s.e.m.) for each of the doses in the effect study. MC samples (a complete set of all parameters) were first extracted from the population parameter statistical distributions of the pharmacokinetics model, as obtained from the pharmacokinetic study. These parameters were then used to simulate the corresponding time courses at the sampled time points. The pharmacokinetic model was considered valid if a significant range ($CI_{95\%}$) of the C_{on} and STZ_{on} observations were within the simulated range.

Mechanistic simulations (with no random component) were performed for comparing single time course profiles of total and unbound C_{blood} in the mean control and streptozotocin rat, but after the same dose and up to 120 min. The simulated concentration time course was for a 3.5-min propofol infusion dose of 6 mg kg^{-1} min^{-1}, using the population pharmacokinetic parameters.

Statistical analysis

Biochemical, and Bayes individualized pharmacokinetic parameters were compared among groups with the *t*-test at the $P \leq 0.05$ level in SPSS (SPSS Inc., Chicago, IL).

Results

Table 1 contains the biochemical parameters of all rats. The plasma concentration of glucose in streptozotocin rats was significantly superior ($P < 0.05$) to controls. There was no significant difference in the levels of cholesterol and plasma triglycerides; nevertheless, there was a reduction of albumin in the streptozotocin group ($P < 0.05$). Body weight was different ($P < 0.05$) between streptozotocin rats and controls despite the fact that both groups were kept under the same conditions during the same time period.

As observed in Table 2, the STZ_{on} rats (effect study) required a significantly ($P < 0.05$) smaller total dose, or alternatively a constant infusion over significantly less time, of propofol to reach the onset compared with control rats. The difference was independent of adjustment to body weight. However, the C_{blood} and C_{brain}, observed in both groups at the onset endpoint, did not show significant differences. The unbound fraction in blood was higher in the streptozotocin group ($P < 0.05$).

After a fixed dose of propofol (bolus of 15 mg kg^{-1}), the time to reach the offset did not differ between the STZ_{off} and C_{off} groups. At that time point, equilibrium with tissues had been reached. The C_{blood} and C_{brain}, when comparing two C_{blood} and two C_{brain} at onset and offset separately for streptozotocin and controls, were not significantly different between the two groups. Given that the C_{brain} was different from the actual biophase concentration (Ce) (due to internal disequilibrium within the fatty brain tissue), the onset and offset values were not comparable. The above allows rejection of a pharmacodynamic change.

The corresponding C_{blood} values reached at onset were higher than those measured at offset in both the control and streptozotocin groups (Table 2). This revealed the existence, in both groups, of a delay between C_{blood} and effect site concentration of similar magnitude (with nearly identical differences between concentrations at onset). At offset the same pharmacological effect was reached for all and at that time equilibrium was necessarily reached (Table 2). The same conclusion was reached based on C_{brain}.

The initial noncompartmental analysis showed differences in the Vd_{ss} for controls, which was three-times higher than for streptozotocin and for MRT, four-times

Table 1 Body weight and biochemical parameters in control and experimental streptozotocin rats

Parameter	Control group (n = 30)	Streptozotocin group (n = 30)
Body weight (g)	315.0 ± 50.1	227.9 ± 39.2*
Glucose (mg dL^{-1})	140.7 ± 41.4	612.5 ± 190.6*
Fasting cholesterol (mg dL^{-1})	95.8 ± 17.9	87.0 ± 27.5
Fasting triglycerides (mg dL^{-1})	51.0 ± 18.1	48.9 ± 34.6
Fasting albumin (mg dL^{-1})	35.5 ± 3.7	26.1 ± 6.5*

Values are mean ± s.d. *Significantly different from control group ($P < 0.05$).

Table 2 Pharmacodynamic study: dose, brain (C_{brain}) and blood (C_{blood}) propofol concentrations at onset and offset of hypnotic effect, in control and streptozotocin rats. The unbound fraction percent (fu%) is listed

	Control group	Streptozotocin group
Onset		
Dose (mg)	6.2 ± 1.9	3.9 ± 1.0*
Dose kg^{-1} (mg kg^{-1})	21.1 ± 4.8	17.4 ± 2.5*
C_{brain} (μg g^{-1})	35.0 ± 8.7	38.0 ± 15.6
C_{blood} (μg mL^{-1})	18.8 ± 4.3	19.7 ± 7.2
Offset		
Sleep time (min)	14.4 ± 3.3	14.8 ± 2.3
C_{brain} (μg g^{-1})	4.3 ± 1.0	3.4 ± 0.1
C_{blood} (μg mL^{-1})	3.6 ± 1.3	4.0 ± 0.8
fu (%)	0.92 ± 0.22	1.37 ± 0.57*

Values are mean ± s.d. *Significantly different from control group ($P < 0.05$).

higher for the controls. There was no appreciable difference between CL for control and streptozotocin groups. Overall two kinetic behaviours were observed. The AUC values for control and streptozotocin did not show differences (129.6 vs 119.8 mg min L^{-1}, respectively).

Subsequently, compartmental analysis of the individual rat pharmacokinetics confirmed the different kinetic behaviour between groups. The time course of C_{blood} in the controls was best modelled by three-compartment disposition kinetics, while the streptozotocin group adjusted best to a two-compartment model. After observing this dichotomy in the pharmacokinetics, a statistical distribution mixture model as implemented in NONMEM was used when treating the two groups jointly, as a single population. Mixture modelling allows the evaluation of possible multi-modalities in the pharmacokinetic parameter distributions, partitioning the population into two (or more) modes and specifying the relative frequency for each mode. Large differences were observed in the central and peripheral volumes of distribution in either two or three-compartment models with nearly 50% partition into the two modes of the population. Consequently, the two groups were considered as samples from different populations and were analysed separately with mixed effects modelling.

The typical population pharmacokinetics parameters (θ) and parameter and residual dispersion measures (ω, σ, respectively) expressed as CV percent are listed in Table 3 for controls and streptozotocin rats. The population model fits are overlaid on the observations in Figure 1 for both groups (Figure 1A, controls and Figure 1B, streptozotocin).

The central volume of distribution (Vd_1) and systemic clearance (CL) were extensive in both groups. V_2 was higher in the streptozotocin group, but this group lacked a third compartment. Vd_{SS} was obtained as sums over empirical Bayes (individual) volume estimates ($Vd_{ss} = Vd_1 + Vd_2 + Vd_3$ for controls and $Vd_{ss} = Vd_1 + Vd_2$ for streptozotocin). Vd_{ss} was again larger in the controls ($P < 0.05$), but there were no significant differences in the population value of CL between the populations (Table 3). The inter-animal variability of clearance was much higher in the streptozotocin group (17.26% in controls vs 61.72% in streptozotocin). There was also a large difference between the residual intra-individual variability between controls and streptozotocin (9.52% vs 32.25%, respectively). The latter, as confirmed by the shape of the residual scatter, was related to the variability in the structure of the pharmacokinetic model within the streptozotocin population.

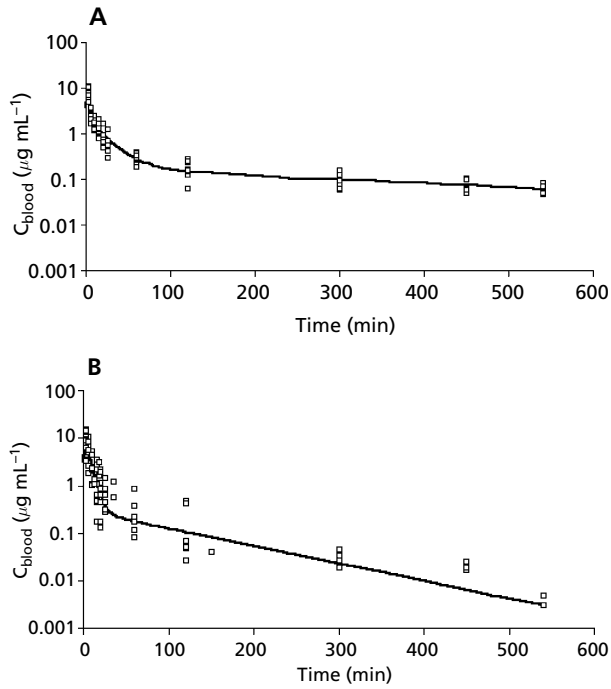

Figure 1 Time course of propofol blood concentration (C_{blood}) observations (□) and best population fits (–) with a three-compartment model for control rats (A) and a two-compartment model for streptozotocin rats (B).

Table 3 Population pharmacokinetics parameters ($\bar{\theta}$) of propofol in control (n = 8) and experimental diabetes mellitus (streptozotocin) rats (n = 10) with standard errors of the estimates (s.e.e.). Intra-individual (σ) and inter-individual (ω) variabilities are expressed as coefficient of variation percent (CV%). Statistical comparison was between model independent parameters, CL and Vd_{ss} The Vd_{ss} and its s.d. was calculated from empirical Bayes estimates of individual rat parameters post population analysis. The model independent parameters CL and Vd_{ss} were compared between groups

Parameters	Control group			Streptozotocin group		
	$\bar{\theta}$	(s.e.e.)	CV%	$\bar{\theta}$	(s.e.e.)	CV%
Vd_1 (L)	0.255	(0.024)	17.46	0.199	(0.030)	44.94
Vd_2 (L)	0.499	(0.091)	56.56	0.827	(0.131)	–
Vd_3 (L)	6.32	(1.18)	39.37	–	–	–
CL (L min^{-1})	0.026	(0.001)	17.26	0.025	(0.005)	61.72*
Q_2 (L min^{-1})	0.053	(0.009)	50.39	0.010	(0.002)	41.95
Q_3 (L min^{-1})	0.028	(0.004)	38.98	–	–	–
σ (CV%)	9.52%	28.47%	–	32.25%	33.65%	–

Control group $Vd_{SS} = Vd_1 + Vd_2 + Vd_3 = 7.67 \pm 2.67$ L vs streptozotocin group $Vd_{SS} = Vd_1 + Vd_2 = 1.11 \pm 0.09$ L; $P < 0.0001$. *$P = 0.5$.

The $CI_{95\%}$ intervals for C_{on} and STZ_{on} observations from the effect study were within the range of expected concentrations after MC simulation from the pharmacokinetic priors and for each of the doses in the effect study group. As an example, the simulation for STZ_{on} is shown in Figure 2.

Table 4 lists the unbound compartmental pharmacokinetic parameters (means from empirical Bayes individualization) corresponding to the unbound C_{blood}. The unbound Vd_{ss} (Vd_{ssu}) was statistically different and lower in the streptozotocin group. There was no difference in CL_u between the control and streptozotocin groups.

Figure 3 shows a mechanistic simulation of the mean C_{blood} profiles (total, Figure 3A; unbound, Figure 3B) for propofol in the control and streptozotocin groups after the same dose (6 mg kg^{-1} min^{-1} for 3.5 min: the mean dose

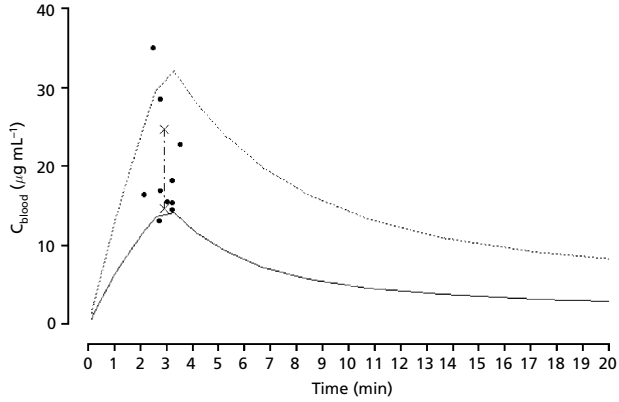

Figure 2 Validation of the pharmacokinetic model in streptozotocin rats. The expected mean C_{blood} time course (–) and +95% interval (- -) are shown. The time – C_{blood} pairs from the STZ_{on} (effect study) are overlaid (•) with their $CI_{95\%}$ (×–×).

Table 4 Unbound pharmacokinetic parameters of propofol in control and streptozotocin groups obtained by dividing empirical Bayes parameter estimates by the fu. Statistical comparisons were between model independent parameters (CL_u and Vd_{ssu})

Parameters	Control group	Streptozotocin group
Vd_{1u} (L)	27.4 ± 3.9	15.4 ± 6.4
Vd_{2u} (L)	61.7 ± 30.2	65.5 ± –
Vd_{3u} (L)	745 ± 290	–
CL_u (L min^{-1})	2.85 ± 0.49	2.15 ± 1.56^{ns}
Q_{2u} (L min^{-1})	5.91 ± 2.63	0.83 ± 0.29
Q_{3u} (L min^{-1})	3.62 ± 1.34	–

Values are mean ± s.d. $^{ns}P > 0.05$. Control $Vd_{ssu} = 834 \pm 291$ L vs streptozotocin $Vd_{ssu} = 81 \pm 6$ L; $P < 0.0001$.

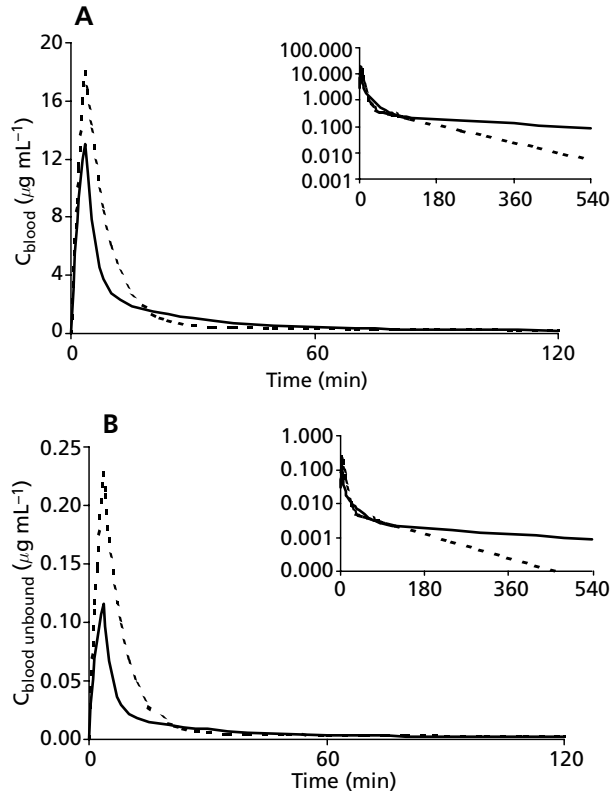

Figure 3 Simulations of total (A) and unbound (B) propofol blood concentration (C_{blood}) time course after a 3.5-min infusion of 6 mg kg^{-1} min^{-1} in controls (–) and streptozotocin (---) rats. The inserts are semilog plots of the same time course.

required for loss of righting reflex, onset in controls). The corresponding total and unbound pharmacokinetic parameters were used. The concentrations were higher in the streptozotocin rats early on, equalizing around 15-min post-dose, and then later becoming smaller in the streptozotocin group compared with the control group. This was in agreement with the similar CL and AUC for the two groups.

Discussion

The variability in the kinetics and dynamics of propofol has been studied extensively in man, in attempts to explain the observed wide range in the hypnotic dose of this drug. Population methodologies have been used and have proven particularly useful in identifying some of the patient-specific covariates involved in the pharmacokinetics and pharmacodynamics, mainly sex, age, weight, cardiac output and comedication (opioid analgesics and benzodiazepines) (Kataria et al 1994; Schnider et al 1998; Schuttler & Ihmsen 2000). Nevertheless, there are few studies (Knibbe et al 2002; Servin et al 2003) where this approach has been used to resolve the variability in the dose or response of propofol in pathologies, in man or in animal models. Here, we have characterized the dose–concentration-effect at onset and offset of propofol in controls and in rats with experimental diabetes mellitus (streptozotocin rats), based on the protocol described by Danhof & Levy (1998), with the aim to identify possible disease related changes.

Streptozotocin rats required a significantly lower dose (or less infusion time) of propofol, compared with the controls, to reach the same pharmacological effect. This could be attributed to alterations in the pharmacokinetics and/or the pharmacodynamics. However, the fact that C_{blood} and C_{brain} measured at the time of loss of the righting reflex (onset) were not different compared with the controls suggested that there were no changes in the pharmacodynamics, since a specific effect (onset of sleep) corresponded to a single concentration in blood or brain. In the recovery of the righting reflex experiment (offset), both controls and streptozotocin rats, receiving a fixed dose of propofol (15 mg kg^{-1} bolus), also had offset at similar C_{blood} and C_{brain}, although quantitatively different to the corresponding value at onset. We recall that due to the liposolubility of propofol C_{brain} is in disequilibrium with the concentration in the biophase, thus not coincident, so the concentrations at onset and offset would not

be expected to be the same. At offset there was distribution equilibrium between biophase, brain and blood, which confirmed the lack of disease-related alterations in the pharmacodynamics. Interestingly, the sleep time to offset did not differ between the two groups, apparently suggesting no change in the pharmacokinetics. Nevertheless, since recovery from hypnosis was achieved when a minimum C_{blood} was reached, this C_{blood}–offset could be reached at the same time via two different kinetic yet intersecting concentration–time courses.

Here, the C_{blood} measured at offset in the controls was similar to that in blood (Cockshott et al 1992) or at the effect site (De Paepe et al 2000, 2001). This concentration was much lower than that required to produce onset, which points to the lack of equilibrium between C_{blood} and C_{brain} in both groups at induction; hence, the equilibration rate (k_{e0}) was also likely to have similar magnitudes in both groups.

At onset, the dose/C_{blood} ratio (an indication of drug distribution) was significantly less in the streptozotocin animals, which suggested a change in the pharmacokinetics. The kinetics were then studied in control and streptozotocin groups after the same dose of propofol (i.v., 6 mg kg^{-1} min^{-1} over 2 min).

The time courses of propofol C_{blood}, in animals from both groups, differed (Figure 2). Noncompartmental analysis showed appreciable Vd_{ss} but no difference in the CL, hence neither in the AUC (CL = dose/AUC). Preliminary individual rat pharmacokinetics fitted as well as joint analysis of all observations, from streptozotocin and controls, with population distribution mixture models, which also verified the existence of distinct pharmacokinetic parameter distributions (populations), for the two groups. Each group was subsequently treated as a separate population.

In NONMEM, three-compartment representation proved optimum for the controls while the pharmacokinetics in streptozotocin rats corresponded to a two-compartment description. Consequently, direct comparisons were between model independent individual rat parameters (CL, Vd_{ss}). In the control rats, central compartment Vd_1, was elevated (0.255 L), exceeding extracellular water volume (in a 250 g rat, Vd_1 is 7.8 mL). Blood CL was high also, close to the total hepatic flow of the rat (0.02 L min^{-1}). (Propofol is known to be a high extraction drug with a hepatic extraction ratio of 1.) All pharmacokinetic parameters in the controls were in agreement with those described in the literature (Cockshott et al 1992; Dutta et al 1998). Additionally, we observed large inter-animal coefficients of variation in some pharmacokinetic parameters (Q_2 and Vd_2), although for Vd_1 (17.5%) and CL (17.3%) they were less than those described by other authors for rats (Dutta et al 1998).

The structural pharmacokinetic change in streptozotocin rats reflected important alterations in the ADME processes with respect to the controls, especially at distribution level, which could in principle be related to the unbound fraction (fu).

The propofol fu in blood was 49% higher in streptozotocin rats than in the controls, possibly related to the lower levels of albumin in streptozotocin. Modification of drug binding in diabetes has been previously documented and could be attributed to the quantitative alteration in albumin and/or free fat acids, but also to a qualitative alteration secondary to a process of glycosylation, as has been suggested by Ruiz-Cabelló & Erill (1984). However, here, glycosylation of proteins was rejected as a possible mechanism since the unbound fraction of propofol was unaffected after a week-long incubation of plasma with high levels of glucose (data not shown).

Changes in the binding of drugs to proteins could be an important source of variability in the kinetics. Further, it is accepted that an increase in the fu would result in an increase of the therapeutic effect since the drug would be more available in the biophase. Nevertheless, this general principle may not be valid for all drugs since highly liposoluble agents with CNS sites of action, like propofol, can access these sites while bound to blood protein (Dutta et al 1997).

In general, an increase in the fu leads to a proportional increase in the volume of distribution (Vd), according to the relation (Rowland & Tozer 1995) $Vd = Vd_p + Vd_t\ fu/fu_t$, where Vd_t and fu_t are the volume and the unbound fraction, respectively, in tissues, and Vd_p is volume in plasma. This is particularly so for drugs with a wide Vd such as propofol. Nevertheless, this increase was not observed in the streptozotocin rats, where paradoxically both Vd and Vd_{ss} were reduced, possibly due to a reduction in the apparent space where the unbound drug was assumed to distribute (Vd_{1u} and Vd_{ssu}, the intrinsic parameters). Specifically, the Vd_{ssu} (comparable parameter) was 10-times lower in streptozotocin. It was not unlikely that this was associated with the pathology, independently of the fu, for example due to a different distribution of water/fat in body or microvasculature changes as suggested earlier (Michel et al 1992; Preston & Epstein 1999). It was the change in these intrinsic parameters (Vd_{ssu}) that was the cause of the observed differences in the dose–effect relationship between groups. Notably also, the weight-corrected Vd values were still significantly different between the groups, so the change in Vd was not related solely to weight.

For dose adjustment, systemic CL was also important. In diabetes, alterations exist in hepatic metabolism and particularly conjugation (Vega et al 1993; Izumi et al 1997), although the final influence on CL depended on the drug. Here, no significant difference was observed in CL between streptozotocin animals and controls. This was not surprising since for propofol the CL was higher than the hepatic flow, hence independent of binding and of intrinsic enzyme changes.

The validated pharmacokinetic parameters were used to simulate the total and unbound C_{blood} after the same dose in the streptozotocin group and control group. The resulting C_{blood} values were above those in controls at the initial times, where the hypnotic effect was usually achieved. Here, the importance of characterizing the total and unbound pharmacokinetics becomes evident, even for drugs whose concentrations (total or unbound) were not in instantaneous equilibrium with the biophase.

Otherwise, the alterations in the kinetics would remain largely unexplained.

Additionally, the study of combined pharmacokinetics and pharmacodynamics can further explain paradoxical observations in the effect vs time profiles, i.e. that besides different pharmacokinetics, both populations recovered from hypnosis at the same time and with similar C_{blood}. Observing the kinetic profiles, a wrong conclusion regarding duration of the effect would have been reached, which would appear longer in the controls (Figure 1). Similarly, the MRT, a parameter indicating the average residence time for drug molecules in the body, was also different between the two groups (41 min in streptozotocin group vs 260 min in the controls). This could again lead to a wrong conclusion concerning time of sleep. Here, in the effect study the C_{blood} and the time at awakening was measured and was similar in controls and the streptozotocin group. Simulations of a fixed dose to a rat from each group verified that at the time of awakening, both populations had coincident concentrations (Figure 3).

In conclusion, the altered and less complex pharmacokinetics of propofol in streptozotocin rats explained the lower dose needed for induction of anaesthesia in these rats. The study also pointed to the importance of performing both pharmacokinetics and pharmacodynamics for obtaining an appropriate adjustment of the dose.

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