

Research Paper

Modeling Methadone Pharmacokinetics in Rats in Presence of P-glycoprotein Inhibitor Valspodar

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Purpose. To quantify the *in vivo* role of P-glycoprotein (P-gp) in the pharmacokinetics of methadone after intravenous and oral administration, using valsopodar as a P-gp inhibitor.

Materials and Methods. Methadone plasma concentrations after intravenous (0.35 mg/kg) and oral (6 mg/kg) administration were analyzed, in absence and presence of valsopodar, using nonlinear mixed effects modeling (NONMEM V). Non-parametric bootstrap analysis and posterior predictive check were employed as model evaluation techniques.

Results. The pharmacokinetics of methadone in the rat was successfully modeled using a two-compartmental model with a linear elimination from the central compartment and a first-order absorption process with lag time. Valsopodar increased methadone *F* by 122% (95%CI: 34–269%) and decreased the *V_c* and *V_p* by 35% (95%CI: 16–49%) and 81% (95%CI: 63–93%), respectively. No effect of valsopodar on other pharmacokinetic parameters was discernible. The non-parametric bootstrap analysis confirmed the absence of bias on the parameter estimates, and visual predictive check evidence the adequacy of the model to reproduce the observed time course of methadone plasma concentrations.

Conclusion. Valsopodar increased methadone's bioavailability as consequence of P-gp inhibition, which resulted in an increased analgesic effect of methadone.

KEY WORDS: bootstrap analysis; methadone; NONMEM; oral absorption; P-glycoprotein inhibition; population pharmacokinetics; Sprague Dawley rat.

INTRODUCTION

Methadone is an opiate drug extensively used in the treatment of acute and cancer pain, either as a primary modality or as an alternative treatment in an opioid rotation protocol (1,2). Besides, methadone is also the cornerstone of opiate addiction therapy, with documented efficacy in preventing opiate abstinence syndrome and in reducing mortality and the use of illicit drugs (3–5).

Some pharmacokinetic features, including acceptable oral bioavailability, minimally active metabolites and a long half-life, which allows a daily dosing regimen, have placed methadone in the current therapeutic position. But this positive features collide with the high unpredictable interindividual variability observed in both the pharmacokinetic and pharmacodynamic processes (6,7), which could be associated with the P-glycoprotein (P-gp) interaction.

The P-gp, also called ABCB1, is a 170 kDa glycosylated membrane protein that was firstly isolated from colchicines-resistant Chinese hamster ovary cells in 1976 (8). Over-expression of P-gp was initially associated with the multidrug resistance of cancer cells, due to its membrane transporter activity that extrudes chemotherapeutic agents out of cells. Nowadays, numerous studies suggest that P-gp's principal role in physiologic status is to protect the organism from toxic substances (9,10). It is known, that this efflux pump is present mainly in the epithelial cell of the gastrointestinal tract and also in other tissues including the renal proximal tubule, the canalicular surface of the hepatocyte and the endothelial cell surface comprising the blood brain barrier (11–13).

During the last decade, a large number of studies have demonstrated the role of P-gp in the absorption of xenobiotics or endogen molecules and, also in the access to certain protected tissues such as brain. In addition, this activity has been also demonstrated for natural and synthetic opiate analgesics (14). In previous studies, our research group showed the active role of P-gp on methadone analgesia, when the opiate was administered, both by intravenous (i.v.) or oral route (p.o.) with a selective inhibitor of P-gp, valsopodar (15). At the same time, another study showed that the administration of quinidine, a non selective P-gp inhibitor, increased the analgesic effect of methadone, in healthy volunteers (16). These studies confirm participation of P-gp in methadone's response, but do not clarify the nature of this interaction in

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terms of pharmacokinetics, neither describe the high variability observed for oral bioavailability (6,17).

Valspodar (or PSC833), a cyclosporin derivative, is an extensively used multidrug resistance modulator. Although its inhibitory activity has been described for others transporters (18), valspodar is considered highly effective in selectively reversing P-gp-mediated multiple drug resistance activity (19), and also in inhibiting the physiological function of P-gp in various tissues, including the intestine, brain, kidney and liver. Studies to elucidate the mechanism of action of valspodar indicated that its potency and specificity might be due to its high affinity for P-gp and the inhibition of P-gp ATPase activity (20,21).

The aim of the present study is to analyze the *in vivo* role of P-glycoprotein in pharmacokinetics of methadone after i.v. and oral administration, using valspodar as a P-gp inhibitor.

MATERIALS AND METHODS

Chemicals. Valspodar was kindly supplied by Novartis Pharma (Basel, Switzerland) and was dissolved in a vehicle containing 5% Cremophor EL (a derivative of castor oil and ethylene oxide; Sigma Aldrich, Steinheim, Germany), 1% ethanol, and 94% saline. Methadone (racemic mixture) chlorhydrate was supplied by Alcaciber (Madrid, Spain).

Animals and Surgical Procedure. Sprague Dawley rats were used in the experiments. Animals were maintained under laboratory standard conditions on a 12 h light/dark cycle, with light from 8:00 A.M. to 8:00 P.M., in a temperature ($21 \pm 2^\circ\text{C}$) and humidity (70%) controlled room. Animals were acclimated a minimum of 4 days before experiments were performed. Food (standard laboratory rat, Panlab, Barcelona, Spain) and water were available ad libitum.

The day before the experiment, rats were lightly anaesthetized with ether. A polyethylene catheter (0.3 mm internal diameter; 10 cm length; Vygon, France) was implanted in the right carotid artery for blood sample collection. Another catheter was inserted into the right jugular vein for the i.v. drug administration. To prevent clotting and catheter obstruction, all catheters were filled with a solution containing NaCl 0.9% (Panreac, Barcelona, Spain) and 1% heparin (50 IU/mL/1, Chiesi Wassermann, Barcelona, Spain). The catheters were tunneled under the animal skin and externalized on the dorsal surface of the neck. The skin in the neck and throat was stitched with normal suture. After the surgery, all rats were kept under fasting conditions but with ad libitum access to water.

The protocols of the studies were approved by the Committee on Animal Experimentation of the University of the Basque Country and the research was adhered to the Principles of Laboratory Animal Care. All animals were treated humanely, in accordance with European Council Directive of November 24, 1986 (86/609/EEG), the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and the conditions specified in the Guide for Care and Use of Laboratory Animals.

Treatment Groups and Pharmacokinetic Sampling. According to methadone administration route, the animals were distributed in two groups, oral (p.o.) and intravenous

(i.v.). Subsequently, each of these groups was also split into two subgroups according to the administration of vehicle or valspodar. In the oral group ($n=38$), animals received methadone 6 mg/kg through a gavage system, which administrate the drug directly into the stomach. In this group, 16 rats received vehicle, while 22 rats received valspodar. In the i.v. group ($n=84$), animals received methadone 0.35 mg/kg i.v. in 30 s, dissolved in saline solution. In this group, 64 rats received vehicle, while 20 rats received valspodar. In this group, the number of rats receiving vehicles was larger as data ($n=52$) from a separate study were pooled.

The i.v. and p.o. methadone doses were selected because previously they were found to be the equipotent doses that produce 50% of the analgesic effect for each administration route (15,22). In the valspodar groups, the P-gp inhibitor (10 mg/kg) was also infused i.v. to rats (23) over 5 min, 30 min before the administration of methadone, using an infusion pump (Kd Scientific).

Blood samples were collected in heparinized tubes from the catheter implanted in the right carotid. After p.o. administration, samples were collected at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180 and 240 min. After i.v. administration, samples were collected at 1, 2, 3, 5, 10, 15, 30, 60, 90, 120, 150 and 180 min. Six alternating sampling schemes were implemented in order to ensure at least three plasma concentrations of methadone per sampling time point. Blood samples were centrifuged at 2,500 rpm for 15 min at 37°C . Plasma was kept frozen at -20°C until assay of methadone. A volume of 1 ml of physiologic serum was used to replace the blood volume after each blood sample was collected.

Methadone Assay. Plasma methadone concentrations were determined by high-performance liquid chromatography (HPLC) according to a previously published method, with minor modifications (24). Briefly, the HPLC equipment included a pump (type 422, Kontron Instruments, Bilbao, Spain) and an ultraviolet (UV) detector (Waters 486, Waters Corp, Milford, MA, USA). The separation was performed in a 5 mm silica column (Apex I, 25_0.45 cm, Teknochroma, Barcelona, Spain) and a Corasil Type II silica precolumn (Teknochroma, Barcelona, Spain). Methadone and the internal standard (benzhexol) were determined by a UV detector at a wavelength of 215 nm.

One milliliter of plasma was mixed with 10 μl of a solution containing the internal standard (1 mg/ml) and 0.5 ml of sodium carbonate buffer (1 M, pH 10) to which 5 ml of n-butyl chloride saturated in water were added, for the extraction of methadone. The mixture was mechanically shaken for 15 min and then centrifuged at 4,000 rpm at 4°C for 10 min. The n-butyl chloride upper layer was vacuum-aspirated and placed in a 5 ml glass tube and evaporated at 50°C (AES 1000, Speed Vac Concentrator, Savant, Spain).

The solid residue was dissolved in 110 μl of methanol, and 100 μl were injected into the chromatograph system. The mobile phase was methanol/1,2-dichloroethane/isopropanol/ammonium perchloride aqueous solution (100 g/l) in the proportion 90.5/5/4/0.5 V/V, at a flow rate of 2 ml/min.

The lower limit of quantification of the assay was 5 ng/ml. The coefficient of variation for the intra- and interassay precision of quality controls was lower than 9 and 7.6%, respectively, for the range of concentrations evaluated that include up to 1000 ng/ml.

PHARMACOKINETIC MODELING

Software. Nonlinear mixed-effects modeling using the first order conditional estimation (FOCE) method was implemented with the NONMEM V level 1.1 software package (GloboMax, Hanover, MD, USA) (25). Compilations were achieved using Microsoft Compact Visual Fortran (version 6.5.0). Graphical and all other statistical analyses, including evaluation of NONMEM outputs, were performed using S-Plus 2000 Professional Release 2 for Windows (Insightful, Seattle, WA, USA).

Population Pharmacokinetic Model. Based on previous population pharmacokinetic analysis of methadone in rats (22,26), an open two-compartment pharmacokinetic model with linear elimination from the central compartment was used to describe the disposition of methadone in rats. The absorption after p.o. administration was characterized by a first-order process with lag time. To fit simultaneously i.v. and p.o. data, the absolute bioavailability was added to the model. During model development, absolute amount of methadone's dose, but not the dose normalized by body weight, was used in order to avoid the confounding effect that a potential body weight effect on pharmacokinetic parameters could have during the covariate analysis. The methadone pharmacokinetic model described above was considered to be the reference model and the following parameters were estimated: systemic clearance (CL), volume of the central compartment (V_c), peripheral volume of distribution (V_p), the intercompartmental exchange flow (Q), lag time (T_{lag}), the first-order absorption rate constant (K_a) and the bioavailability (F).

A 50-fold difference in methadone plasma concentrations has been previously observed and strongly suggested the need for a heteroscedastic residual error. As a consequence, the measured plasma concentrations and model predictions were transformed into natural logarithms, and the magnitude of the residual variability in the log transformed concentrations was modeled using an additive error model (27). The interanimal (IAV, between animals) variability in the model parameters were assumed to follow the lognormal distribution.

During the initial model development process, interindividual random effects were added to all model parameters, assuming no correlation between parameters. Therefore, the interindividual random effects that turned out to be negligible were kept in the model, that is, not fixed to zero, because the incorporation of covariate effects into the model may affect the random effects estimates, as suggested previously (28).

The feasibility of a one compartmental model and/or the inclusion of a lag time were tested and compared to the reference model to select the optimal structural model for the characterization of the methadone pharmacokinetics. For hierarchical models, the improvement in the fit obtained through the inclusion of a fixed effect into the overall model was assessed using the likelihood ratio test LRT (29).

Covariate Analysis. After fitting the reference model, empirical Bayesian estimates of the individual pharmacokinetic parameters were computed to screen the influence of valspodar, body weight, plasma albumin, α_1 -acid glycoprotein on methadone pharmacokinetic parameters. These covariates has been selected as potential predictors of methadone pharmacokinetics in rat (6). The covariate screening analysis

was based on visual graphical inspection and stepwise linear regression of the relationships between the individual Bayesian pharmacokinetic parameters and the covariates. Based on this analysis, covariates with statistically significant effect ($p < 0.05$) on pharmacokinetic parameters were evaluated for being incorporated into the population model following a forward-inclusion and backward-elimination processes, as previously described (29). Valspodar effect was incorporated into the structural model as index variables, whereas other covariates were incorporated as quantitative variables centered around the median and using a power equation.

Model Refinement. At this stage, the interindividual random effects that turned out to be not estimable in the prefinal model were fixed to zero. The distributions of the estimable random effects were graphically examined to evaluate the normality assumption. In addition, each random effect was also plotted against all other random effects to explore the potential correlations. The random effects with the highest correlations were tested by including the corresponding non-diagonal element in the matrix of random effects. If the implementation of a correlation between random effects significantly improved the fit, the off-diagonal element in the matrix of random effects was kept in the model and the process was repeated until no further improvement of the fit could be achieved. At this stage, the final population pharmacokinetic model was obtained.

Model Validation. A non-parametric bootstrap analysis was performed as an internal model evaluation technique, using the package Wings for NONMEM (N. Holford, Version 4.04, June 2003, Auckland, New Zealand). A new replication of the original dataset (a bootstrap sample) was obtained by N random draws of individual animal data (with replacement) from the original dataset. The final population pharmacokinetic model was re-fitted to each new dataset and this process was repeated 1,000 times with different random draws. The stability of the final model was evaluated by visual inspection of the distribution of the model parameter estimates from the new datasets and compared with that obtained from the fit of the original dataset (30). Bootstrap runs with unsuccessful minimization were excluded from further analysis. The final model parameter estimates were compared to the median and 95% confidence intervals of the non-parametric bootstrap replicates of the final model. The 95% confidence intervals were derived from the bootstrap percentiles.

A visual predictive check was performed (31). The parameter estimates obtained from the final model were used to simulate the population pharmacokinetic profile after the administration of methadone 0.35 mg/kg i.v. and 6 mg/kg p.o. A nonparametric 95% prediction interval around the median plasma concentration was constructed to quantify the (inter-animal and residual) variability in the model predictions, and to visually compare with the observed methadone plasma concentrations.

RESULTS

A total of 122 Sprague Dawley male rats were available for this analysis. The mean (SD) of body weight in absence and presence of valspodar were 239.99 g (23.54) and 238.78 g (15.14), respectively. Also, mean (SD) albumin in absence and

presence of valspodar were 29.93 g/l (3.71) and 28.15 g/l (3.16), respectively. In addition, mean (SD) α_1 -acid glycoprotein in absence and presence of valspodar were 0.83 g/l (0.31) and 0.84 g/l (0.40), respectively. The similarity of the covariates distribution in the i.v. and p.o. groups allowed the pooled pharmacokinetic analysis and the evaluation of these covariates as potential sources of interanimal variability. Overall, 201 plasma concentrations of methadone, 1.65 plasma concentration per rat, were available for the population pharmacokinetic analysis.

An open two-compartment model with linear elimination from the central compartment and a first-order absorption with lag time (reference model) was successfully fitted to the pharmacokinetic data obtained after i.v. and p.o. administration of methadone in rats. Two-compartment disposition model was preferable to one-compartment model because the Δ MVOF was 19.9. The inclusion of the lag time significantly improved the fit, as the Δ MVOF was 10.5.

The screening analysis of the covariates suggested the effect of the valspodar in all pharmacokinetic parameters of methadone. However, none of the other covariates evaluated evidenced any relationship with methadone pharmacokinetic parameters. Consequently, the effect of valspodar was simultaneously included in all pharmacokinetic parameters of the population model. The resulting full model provided a better fit than the reference model (Δ MVOF: 53.475; df: 7; $p < 0.001$). During the backward elimination process (Table I), the effect of valspodar on CL, Q , T_{lag} and K_a was excluded from the full model because of the lack of statistical significance. Therefore, only the effect of valspodar on V_c , V_p and F were retained in the population model (Model 8), which significantly improved the fit with respect to the reference model (Δ MVOF: 50.895; df: 4; $p < 0.001$) and was similar to the full model (Δ MVOF: 2.58; df: 4; $p: 0.108$).

At this stage, the inter-animal variability of V_c , V_p , Q and K_a was not estimable from the available data, and was therefore fixed to zero. In addition, the uncertainty in the estimation of T_{lag} random effect was large (from 2.9 to 109%) and, as a consequence, it was fixed to 0 as the Δ MVOF, 5.29, suggested limited improvement in the model fit when this random effect was added into the model. The examination of the estimable random effect distributions confirmed the validity of the normality assumption. None of

the correlations between the random effects of CL and F were significant. As a consequence Model 8 was considered the final population pharmacokinetic model. The final estimates of the population pharmacokinetic parameters are presented in Table II. According to the final model, valspodar increased methadone F by 122% (95%CI: 34–269%) and decreased the V_c and V_p by 35% (95%CI: 16–49%) and 81% (95%CI: 63–93%), respectively. No effect of valspodar on other pharmacokinetic parameters was discernible. The inclusion of valspodar effect in the population pharmacokinetic model decreased the magnitude of IIV for F by 32% and the residual variability by 8%, respectively.

The goodness-of-fit plots for the final model are presented in Fig. 1 and showed random uniform scatter around the line of identity and suggested the absence of any trend or bias. Methadone plasma concentrations observed after i.v. and p.o. administration in presence and absence of valspodar and the corresponding model predictions are shown in Fig. 2 as a result for the visual predictive check. The visual analysis of Fig. 2 clearly evidenced the adequacy of the model to reproduce the observed concentration-time profile of methadone in rats after i.v. and p.o. administration, and the effect of valspodar on the methadone's pharmacokinetic profile. Finally, Fig. 3 evidenced the lack of relationship of the other covariates evaluated (body weight, α_1 -acid glycoprotein and albumin) with the methadone systemic clearance and absolute oral bioavailability.

The results of the nonparametric bootstrap analysis further confirmed the absence of bias in the estimation of model parameters. From the 1,000 bootstrap replicates of the model, only 0.02% failed to minimize successfully. Unsuccessful bootstrap replications were considered unreliable and therefore excluded from further analysis. The analysis of the results from the 998 bootstrap replicates, which minimized successfully, is given in Table II. The parameter estimates of the final population pharmacokinetic model were very similar to the median of the non-parametric bootstrap replicates, and all were contained within the 95% confidence intervals obtained from the bootstrap analyses. The precision of the NONMEM parameter estimates was also good, since the relative standard error from the bootstrap analysis for the majority of fixed and random effects was lower than 28 and 50%, respectively. The two exceptions were the estimation of the V_p in presence and

Table I. Backward Elimination Process for Assessing Valspodar Effect on Methadone Pharmacokinetics Parameters

Model	Valspodar Effect	Model Compared	LRT	Degrees of Freedom	<i>P</i>
Full Model ^a	Valspodar effect in all parameters	–	–	–	–
1	On V_c excluded	Full model	13.69	1	<0.001
2	On CL excluded	Full model	3.55	1	0.060
3	On V_p excluded	Full model	7.48	1	0.006
4	On Q excluded	Full model	0.11	1	0.740
5	On K_a excluded	Full model	5.44	1	0.020
6	On T_{lag} excluded	Full model	1.18	1	0.277
7	On F excluded	Full model	30.36	1	<0.001
8 ^b	On CL, Q , K_a and T_{lag} excluded	Full model	2.59	4	0.108
9	On Q , T_{lag} , CL, K_a and V_c excluded	8	11.69	1	0.001
10	On Q , T_{lag} , CL, K_a and V_p excluded	8	27.09	1	<0.001
11	On Q , T_{lag} , CL, K_a and F excluded	8	8.08	1	0.004

^a Full model has a MVOF equal to –63.49.

^b This is the final model and includes the valspodar effects on F , V_c , and V_p .

Table II. Population Pharmacokinetic Parameters of Methadone in Sprague Dawley Rats and the Stability of the Model Parameters Using Non-Parametric Bootstrap Analysis

Model Parameters	Original Dataset	Non-parametric Bootstrap ($N=998$ Replicates)		
	Estimate (RSE%)	Median (RSE%)	95%CI Lower Limit	95%CI Upper Limit
Structural Model				
V_c (L)	1.19 (8.22)	1.19 (8.18)	1.01	1.40
CL (l/h)	0.55 (14.88)	0.53 (18.05)	0.33	0.71
Q (l/h)	1.55 (14.86)	1.58 (15.57)	1.17	2.14
V_p (L)	4.45 (27.87)	4.54 (59.6)	2.86	11.61
K_a (h^{-1})	1.50 (12.27)	1.48 (13.69)	1.10	1.87
T_{lag} (min)	2.45 (39.39)	2.5 (43.35)	0	3.98
F (%)	0.23 (21.21)	0.23 (26.68)	0.15	0.39
Valspodar Effect^a				
Ratio V_c	0.65 (8.76)	0.65 (12.46)	0.51	0.84
Ratio V_p	0.19 (34.15)	0.19 (40.54)	0.07	0.37
Ratio F	2.22 (16.58)	2.20 (27.11)	1.34	3.69
Interindividual Variability (%)				
ω_{CL}	37.95 (92.36)	59.70 (48.30)	0.64	87.91
ω_F^b	0.90 (31.29)	0.92 (19.27)	0.62	1.30
Residual Variability (%)				
Additive error	44.94 (15.54)	43.70 (8.02)	36.47	50.40

^a Expressed as the ratio between pharmacokinetic parameter in presence and absence of valspodar.

^b Standard deviation in the logit domain.

absence of valspodar and the T_{lag} . The lack of precision in T_{lag} could be due to the nature of available data as the rats receiving i.v. methadone do not receive the p.o. formulation, and viceversa. However, since the estimated typical value of

the T_{lag} is shorter than the time to first sample collection, the relevance of this parameter is limited. In addition, bootstrap analysis also confirmed the inclusion of the valspodar effects in the final population pharmacokinetic model according to the

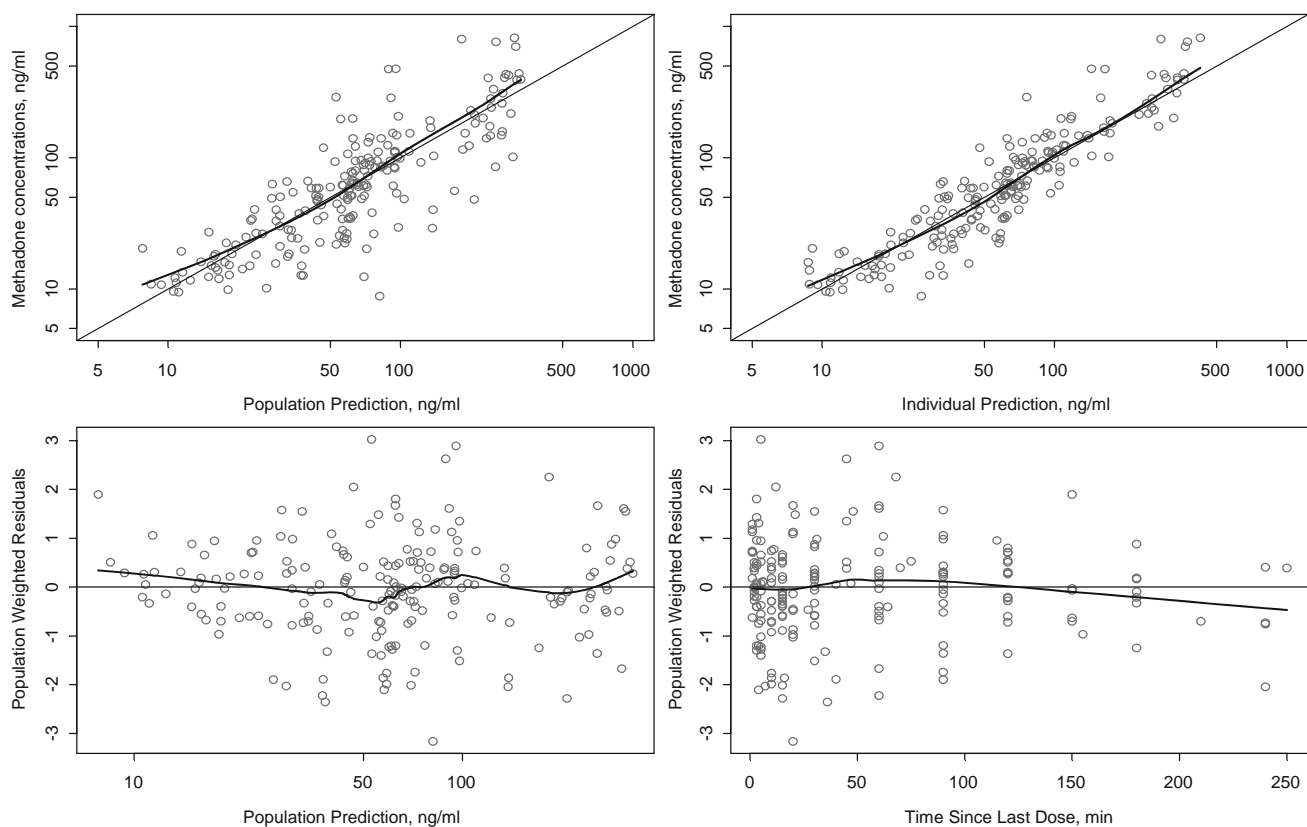


Fig. 1. Goodness-of-fit plots for the final population pharmacokinetic model: scatter plots of the observed plasma methadone concentrations versus the population model predictions (*upper left panel*) and the individual model predictions (*upper right panel*), scatter plots of the population weighted residuals versus the population model predictions (*lower left panel*) and time since last dose (*lower right panel*).

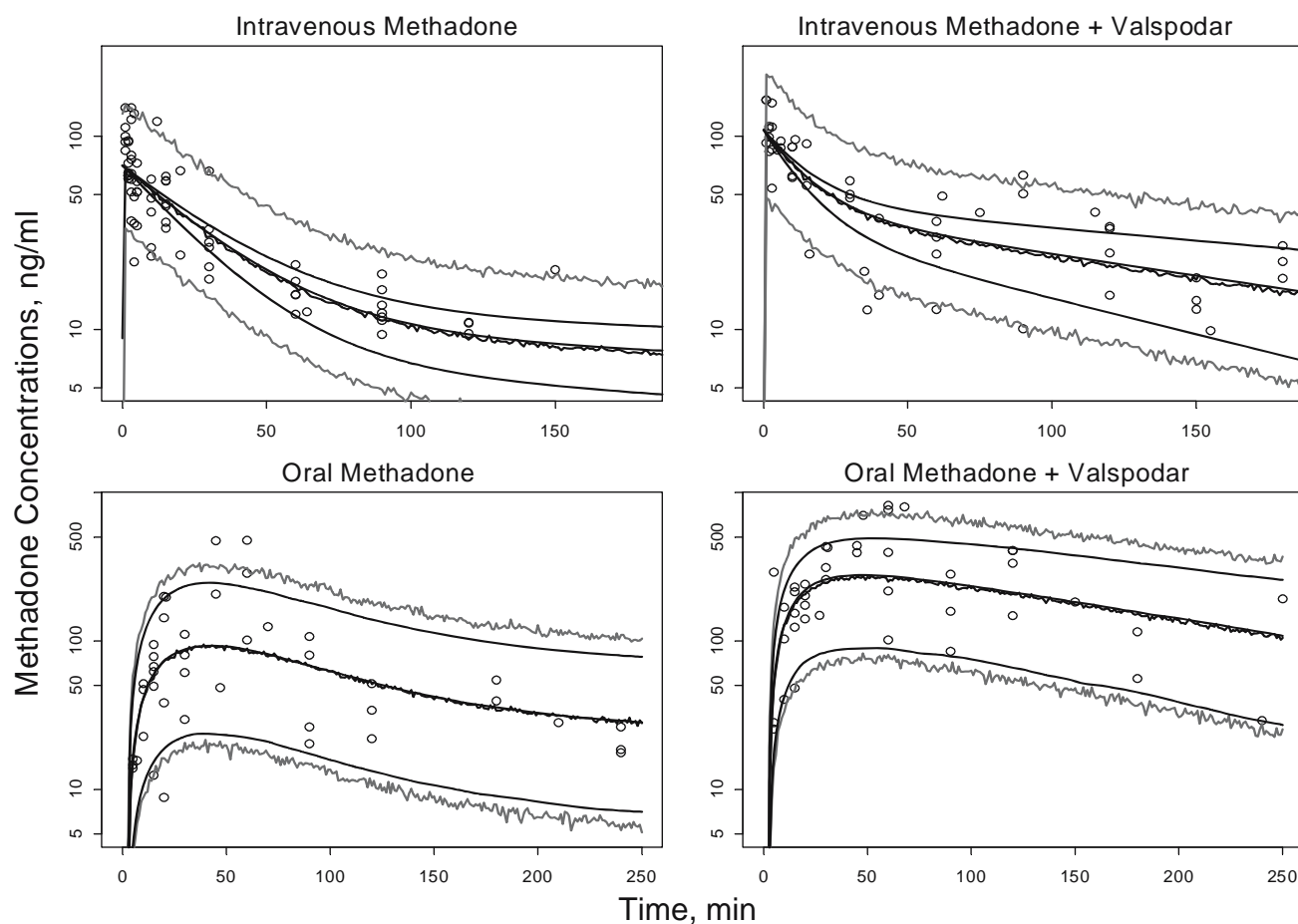


Fig. 2. Time course of methadone plasma concentrations in Sprague Dawley rats and its variability, and model predicted methadone concentrations in plasma. Lines represent the 2.5th, 50th and 97.5th percentiles of the methadone plasma concentration distribution in *grey* and the prediction interval of between subject variability in *black*.

LRT, since the 95% confidence intervals of the valsopodar effect on V_c , V_p and F did not include the null value. Finally, bootstrap analysis suggested a biased in the estimation of the uncertainty in the model parameters, quantified as the RSE, as the results of the bootstrap analysis for random effects revealed that the RSE was much lower than that obtained from the original analysis.

DISCUSSION

In order to estimate the role of P-gp on methadone's bioavailability, a combined i.v. and p.o. pharmacokinetic analysis was conducted using modeling techniques. An open two-compartment pharmacokinetic model with linear elimination from the central compartment and first order absorption with lag time was found appropriate to describe the methadone pharmacokinetics in rats after i.v. and p.o. administration, in presence and absence of valsopodar, a P-gp inhibitor. In absence of valsopodar, a biphasic decline in plasma levels was observed after i.v. administration of methadone, and the pharmacokinetic parameters obtained were similar to those previously reported for other authors (22,26). The simultaneous analysis of both administration routes, allowed us to estimate the absolute oral bioavailability, besides the others

pharmacokinetic parameters, and to evaluate valsopodar's effect on its variability.

However, traditional non-compartmental analysis of rodent pharmacokinetic data requires the pooling of data as the number of samples obtained from each animal is generally insufficient to describe a full pharmacokinetic profile per animal. Such naïve pooled data approach is based on the incorrect assumption that all data come from one and the same animal. As this approach does not take into account the existing variability and uncertainty, the resulting pharmacokinetic information may be inaccurate. In that regard, a population pharmacokinetic approach offers an important advantage over conventional data analysis methods as the former is able to deal with sparse data allowing the estimation of typical population pharmacokinetic parameters (fixed effects), together with their associated variabilities (random effects), in a single step. This technique also allows the possibility to pool data from different studies and objectively evaluate sources of such variability by relating them to some explanatory variables (32,33). For these reasons, population pharmacokinetic approach was selected to analyze the data presented in this paper.

The *in vitro* gut sac model confirmed that methadone is a substrate of P-gp (34), and therefore modulation at this level could contribute to the wide variation observed in metha-

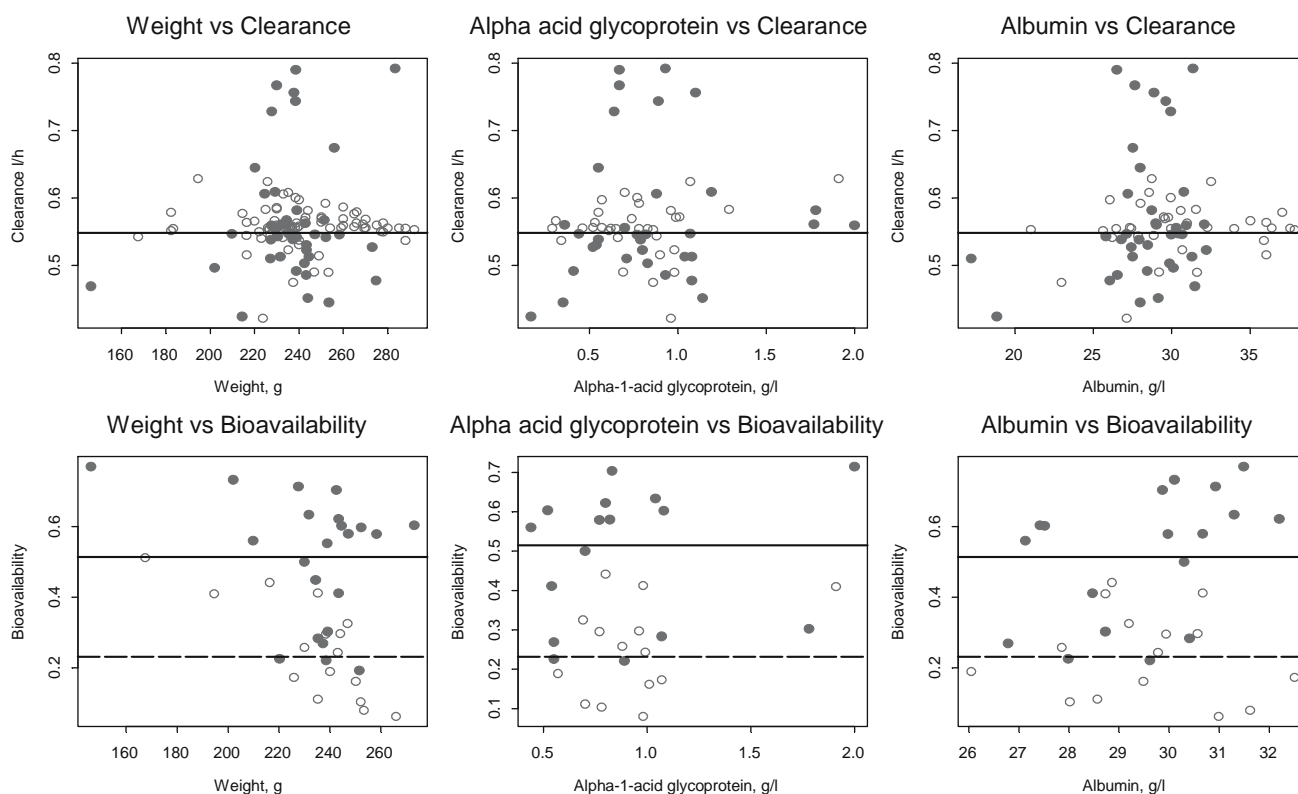


Fig. 3. Relationships of body weight (left panel), α_1 -acid glycoprotein (middle panel) and albumin (right panel) with methadone systemic clearance (upper panels) and absolute oral bioavailability (lower panels). The horizontal lines represent the typical value of model parameters in presence (solid) and absence (dotted) of valsopodar. The dots represent the individual Bayesian estimated of model parameters in presence (black) and absence (white) of valsopodar.

done oral bioavailability. An *in vivo* rat model was used in this study to evaluate the influence of P-gp in the modulation of methadone oral bioavailability using a highly specific P-gp inhibitor, valsopodar. Our results evidence an increase in methadone's oral bioavailability after valsopodar administration, which most likely is due to the valsopodar P-gp inhibition in the gut. Fig. 4 display the model-based distribution of the methadone area under the curve (AUC) and maximum plasma concentration (C_{Max}) after the administration of methadone 0.35 mg/kg i.v. and 6 mg/kg p.o. in presence and absence of valsopodar. The results evidence that the increase in methadone AUC and C_{Max} due to valsopodar is more pronounced after oral administration, as compared to the intravenous administration.

An alternative explanation could be that the increase in methadone F is mediated by valsopodar inhibition of CYP3A4, an enzyme involved in methadone metabolism (6). In fact valsopodar inhibits CYP3A4, but the valsopodar concentrations achieved with the dose used in this study are below the valsopodar concentrations that inhibits 50% of the CYP3A4 activity (23). In fact, after the administration of valsopodar, similar increases in oral bioavailability have been observed for other P-gp substrates such as paclitaxel (35) and ivermectin (36). However, the administration of valsopodar did not modify the pharmacokinetics of drugs like oxycodone that is not P-gp substrate (37) but it is partially metabolized by CYP3A4 (38).

Another potential explanation could be that Cremophor EL, the vehicle of valsopodar, is known to be a modulator of

P-gp (39). However in a previous study (15), our research group demonstrated that Cremophor EL does not modify the analgesic effect after intravenous or oral administration of methadone. Nevertheless, in the current study, the same Cremophor EL dose was administered to the animals that received valsopodar and to those how did not receive it. Consequently, the P-gp inhibition observed in this study was not due to the effect of Cremophor EL, and should be only assigned to the valsopodar activity.

The increase in methadone's oral bioavailability in presence of valsopodar could explain partly the results observed in a previous study, (15) where an increase of the analgesic response to methadone was described in presence of this P-gp inhibitor. However, this phenomenon could be also explained because of valsopodar inhibition of P-gp at blood brain barrier. In theory, the valsopodar inhibition of P-gp at blood brain barrier would result in an accumulation of methadone in brain, which would increase the analgesic response. If no valsopodar effect at the blood brain barrier would be present, a 2.14 fold increase in methadone's oral bioavailability in presence of valsopodar would result in similar increase in methadone's brain concentrations. However, if P-gp inhibition at blood brain barrier would be present, a more that 2.14 fold increase in methadone's brain concentrations would be expected. Previously, a 4 to 6 fold increase in methadone concentrations at brain in presence of valsopodar was reported. (15) Taken together, these findings suggest that the increase in the methadone analgesic response observed after the admini-

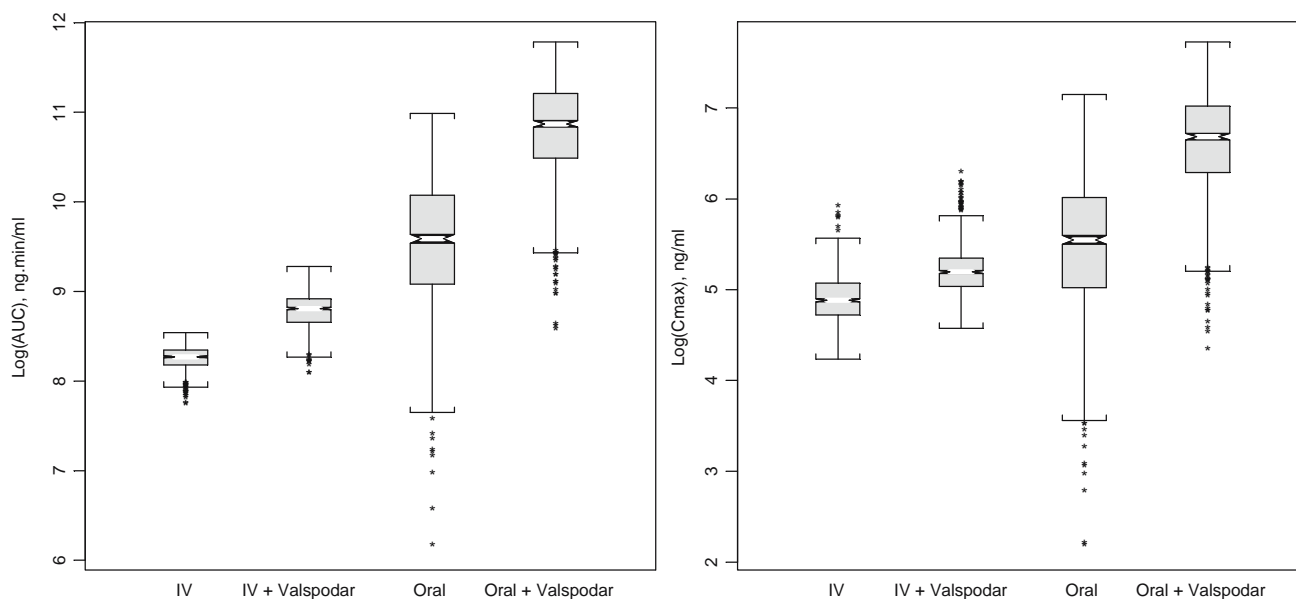


Fig. 4. Model-predicted effect of valsopodar on methadone area under the concentration time-curve (AUC, *left panel*) and maximum plasma concentrations (C_{Max} , *right panel*).

istration of valsopodar could be due to the dual effect of the increase in methadone's oral bioavailability plus the modulation of the methadone brain to plasma distribution, both processes mediated by the P-gp. Discriminating the relative role of P-gp at the intestinal tract and blood brain barrier warrant further investigation through the application of quantitative PK/PD modeling, which should take into account methadone concentrations and the analgesic response after i.v. and p.o. routes of administration, in presence and absence of valsopodar.

Interestingly, although an increase in methadone F was found in presence of valsopodar, no effect of valsopodar on CL , K_{as} , T_{lag} , and Q could be discerned, but a reduction in the V_c and the V_p was observed. Some authors have reported similar changes in the drug distribution for other P-gp substrates such as talinolol (40), docetaxel (41) and doxorubicin (42) in the presence of P-gp inhibitors. The mechanisms responsible for the decreased volume of distribution at steady state (V_{ss}) remain unclear. It could be hypothesized that the inhibition of P-gp might decrease the transport of methadone in lungs from the interstitium into the lumen, and therefore, decrease the V_{ss} . In fact, up to 90% of the i.v. methadone dose is initially sequestered in lung tissue (43). At same time, P-gp is highly expressed in the lung tissue where transports the substrates from the interstitium into the lumen (44). Taken together, these findings could explain the decrease in the V_{ss} , however, the effect of P-gp in other locations as liver and kidney could also participate in methadone V_c and V_{ss} reduction, although the mechanisms responsible are not well understood.

The body weight, plasma albumin and α_1 -acid glycoprotein effect in the pharmacokinetic were also evaluated. Some studies have reported the influence of methadone's free fraction in the pharmacokinetic parameters of methadone (6). However, the effect of albumin concentration in plasma did not influence in the pharmacokinetic parameters as other authors have described previously (45, 6). Although body weight and α_1 -acid glycoprotein have been identified as potential sources of methadone pharmacokinetic variability

(6), our results failed to confirm these findings, probably due to the narrow range of variability for these covariates in our dataset (Fig. 3). In addition, our research group demonstrated in a previous study that valsopodar administration did not modify methadone's free fraction (15).

From the current data, only the interindividual variability of CL and F could be estimated. The remainder of the interindividual random effects was found to be negligible or difficult to quantify them, probably because the data available does not allow their proper estimation. In addition, the variability observed is a mixture of between and within animal variability because only single dose studies were available for the analysis. In order to characterize within animal variability, plasma samples collected from the same animal after two or more dosing occasions should be collected. However, as methadone has been described as an inducer of its own metabolism (6), and continuous exposition to P-gp substrates has also shown to modulate P-gp activity over time (46), additional studies should be conducted with multiple doses of methadone in order to analyze separately these effects and account for interoccasion variability. As only one dose of the methadone after intravenous and oral administration has been evaluated in the current study, further investigation is warranted in order to explore the valsopodar effect on a wider range of methadone doses using single and multiple dose studies. At same time, these studies will help in externally evaluate the model and to overcome the limitations of the internal model evaluation techniques employed in the current analysis.

The potential clinical implication of the findings reported in this paper are derived from the fact that both methadone AUC and C_{Max} are increased by the inhibition of P-gp, which in turn might results in an increased and prolonged analgesic effect as previously suggested (15). Therefore, the therapeutic inhibition of P-gp could be used in the clinical practice to increase the methadone's dose intervals, which might help to control the opiate abstinence syndrome and increase the treatment compliance. However,

this advantage is initially theoretical and further investigation should be performed in the context of clinical trials in order to test if the findings obtained in rats are similar in humans and, consequently, if the inhibition of P-gp results in a more efficacious and safer control of the opiate abstinence syndrome. At the same time, the P-gp effect on methadone's pharmacokinetics should be taken into account during the patient clinical management as the concomitant administration of drugs with P-gp inhibition activity could result in situations with methadone overdosing, while the coadministration of drugs that induce P-gp might result in a lack of methadone efficacy unless its dose is increased.

CONCLUSION

In summary, a population pharmacokinetic approach has been used to integrate the knowledge gathered from pharmacokinetic studies in rats, after the i.v. and p.o. administration of methadone in presence and absence of valsopodar. The methadone pharmacokinetics has been well characterized by a standard two compartment pharmacokinetic model with linear elimination from the central compartment and first order absorption process with lag time. Valsopodar increases methadone's bioavailability by 122% (95%CI: 34–269%), probably as a consequence of the P-gp inhibition, which in turn has been associated with an increased analgesic effect.

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