Chronopharmacology of intravenous and oral modified release verapamil

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Aims Using a stable isotope technique which allows simultaneous and differential measuring of orally and intravenously administered drugs we compared the pharmacokinetics and pharmacodynamics of unlabelled modified release verapamil p.o. (steady state) and deuterated verapamil i.v. (single dose) following morning and evening administration.

Methods Twelve female and 12 male healthy volunteers were studied in a randomized, crossover design. During the last day of each treatment period (day 6 and day 10) pharmacokinetics and pharmacodynamics (PR interval) of verapamil were assessed; 1 h before ingestion of a new R/S-verapamil 240 mg modified release formulation (08.00 h *vs* 20.00 h) a single dose of 10 mg d₇-R/S-verapamil was administered intravenously. Serum levels of unlabelled and labelled R/S-verapamil were measured by gas chromatography/mass spectrometry. In selected samples of serum which were chosen at $t_{\min,po}$ and $t_{\max,po}$ the enantiomers were separated by chiral high-performance liquid chromatography in order to calculate R- to S-verapamil serum concentration ratios.

Results We observed no significant differences in pharmacokinetics (AUC_{po} , $C_{\max}$, $t_{\max}$, CL_o , F and R/S enantiomer ratio) between morning and evening treatment with modified release verapamil and there was no influence of time of dosing on mean prolongation of PR interval. AUC_{iv} , CL , V_{ss} and d₇-R/d₇-S enantiomer ratio following verapamil i.v. did not show circadian variation. $t_{1/2}$ was slightly but statistically significantly increased after the morning infusion. PR-prolongation was significantly greater after verapamil i.v. in the morning than in the evening. The 90% confidence intervals of the differences between morning and evening administration in AUC_{po} , $C_{\max}$ and AUC_{iv} were within the equivalence range of 0.8–1.25.

Conclusions Time of dosing has no significant influence on pharmacokinetics and pharmacodynamics of this new modified release formulation of verapamil. Circadian variation in presystemic metabolism of verapamil was not observed.

Keywords: chronopharmacology, presystemic metabolism, stable isotope technique, verapamil

Introduction

There are numerous investigations demonstrating that pharmacokinetics and drug effects can depend on the time of drug administration and on biological rhythms of physiological functions [1, 2]. For example, rate of drug absorption may be circadian-phase dependent because of delayed gastric emptying time in the evening, however, without major impact on bioavailability [3]. Proven

circadian variation in hepatic blood flow may affect pharmacokinetics of drugs with a high hepatic extraction ratio [4]. The calcium antagonist nifedipine showed significant differences in concentration-time profiles and bioavailability between morning and evening administration of an immediate-release oral formulation, but no differences in systemic clearance following i.v. infusion [5]. In the case of nifedipine which is a substrate of the most abundant cytochrome P450 enzyme present in human gut (CYP3A4) [6] gastrointestinal mechanisms like altered drug absorption or gut wall metabolism may well be involved in chronopharmacokinetics. Since *N*-demethylation and *N*-dealkylation of the calcium channel

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blocker verapamil are also catalysed by CYP3A4 circadian changes in intestinal enzyme activity may affect prehepatic extraction of verapamil. A clinical study in a limited number of patients found circadian variation in the pharmacokinetics of sustained release verapamil with the area under the plasma concentration–time curve and the time to peak concentration being significantly higher after administration at night than in the morning [7]. However, an adequate number of subjects is needed to detect chronopharmacological effects because of considerable intraindividual variation in bioavailability of verapamil on separate occasions [8]. So far there are no data available on circadian variation in systemic clearance and presystemic extraction of verapamil. Therefore, the aim of our study was to compare pharmacokinetics and pharmacodynamics of verapamil after morning and evening treatment in fed healthy volunteers using a stable isotope technique. Simultaneous administration of verapamil by the intravenous and oral route allows the assessment of systemic clearance and absolute bioavailability in one single experiment. Variables influencing chronopharmacology such as intake of meals, posture and activity have been strictly controlled during the trial.

Methods

Subjects

Twelve female and 12 male healthy Caucasian volunteers participated in the study (age 31.3 ± 3.7 years, body weight 66.1 ± 9.1 kg, height 174 ± 7 cm; mean $\pm$ s.d.). Before entering the study each subject underwent a complete medical examination including laboratory investigations, vital signs and ECG. No medication was allowed for 2 months before and during the trial except of oral contraceptives in one woman. Oral contraceptives do not interfere with the metabolism of verapamil [9]. The study was approved by the Ethics committee of the *Landesärztekammer Baden-Württemberg*, Stuttgart, Germany, according to the ethical guidelines of the 1996 Declaration of Helsinki. Written informed consent was obtained from each subject.

Protocol

The study had a randomized, crossover design. For 10 consecutive days the volunteers received once daily a new 240 mg modified release formulation of unlabelled racemic verapamil (R/S-verapamil-HCl, Knoll AG, Ludwigshafen, Germany) orally with 100 ml water. Each subject was randomly allocated either to sequence one starting with the morning treatment (08.00 h) or to sequence two beginning with the evening treatment (20.00 h). After six oral doses of verapamil subjects switched to the other application time. On study day 6

and 10 an intravenous infusion (10 min) of 10 mg deuterated, racemic verapamil (d_7 -R/S-verapamil-HCl, obtained from Knoll AG, Ludwigshafen, Germany) was administered additionally one hour before oral ingestion of modified release verapamil, and blood samples (10 ml) were taken for drug analysis from the contralateral arm before and 10, 15, 20, 30, 40 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 24 and 25 h after the start of infusion. 12-lead ECG (Cardiovit CS-100, Schiller AG, Baar, Switzerland) was recorded in supine rest position with each blood sample. Blood samples were centrifuged for 10 min and thereafter serum was stored at -20°C . On both study days standardized meals (breakfast and supper, respectively) were provided 15 min before the oral application of verapamil. 24 h before and throughout the whole trial neither alcohol, grapefruit, methylxanthine containing foods/beverages nor smoking were permitted.

Analytical method for determination of unlabelled and labelled verapamil

Serum R/S-verapamil and d_7 -R/S-verapamil levels were determined by gas chromatography/mass spectrometry with d_3 -R/S-verapamil (obtained from Knoll AG, Ludwigshafen, Germany) as internal standard according to a published method [10]. The mass spectrometer was operated in the selected ion monitoring mode, using m/z 303 for R/S-verapamil, m/z 306 for d_3 -R/S-verapamil and m/z 310 for d_7 -R/S-verapamil. The lower limit of quantification was 1 ng ml^{-1} for R/S-verapamil and 0.5 ng ml^{-1} for d_7 -R/S-verapamil, using 1 ml of serum. The calibration curve was linear over the whole concentration range from 10 to 500 ng ml^{-1} and $1\text{--}250\text{ ng ml}^{-1}$ for R/S-verapamil and d_7 -R/S-verapamil, respectively. Repeatability (intraday/within-run reproducibility) was determined by evaluation of the results of four different quality control samples (1.0 , 10.0 , 100 and 400 ng ml^{-1} for R/S-verapamil and 0.5 , 1.0 , 20.0 and 200 ng ml^{-1} for d_7 -R/S-verapamil) at least six times on the same day. The coefficients of variation including the limit of quantification were below 5.5% for R/S-verapamil and below 10.5% for d_7 -R/S-verapamil. The accuracy of the method expressed by the bias (deviation of the average of the measured concentration from the amount added) varied between -7.7% and 8.9% for R/S-verapamil and between -11.8% and 12.4% for d_7 -R/S-verapamil. Each day that analyses were performed quality control samples at three different concentrations (10.0 , 100 and 400 ng ml^{-1} for R/S-verapamil and 1.0 , 20.0 and 200 ng ml^{-1} for d_7 -R/S-verapamil) were analysed in duplicate. At least four of the six quality control samples had to be within 20% of their respective nominal values. Coefficients of variation

of R/S-verapamil were below 5.4% for all concentrations measured, the bias observed varied between 9.9% and 12.7%. Coefficients of variation of d₇-R/S-verapamil were below 10.6%, the bias observed varied between -10.5% and -3.8%. A modification of a previously described method was used for separation of the enantiomers in selected samples of serum [11]. In brief, R- and S-verapamil were separated by chiral high-performance liquid chromatography on a Chiralpak AD column (0.46 × 25 cm). Fractions containing R- and S-verapamil, respectively, were collected and subjected to gas chromatography/mass spectrometry as described above.

Pharmacokinetic analysis

Pharmacokinetic calculations were based on serum concentrations above the lower limit of quantification. Using standard noncompartmental methods (TopFit 2.1®) estimates of extrapolated area under the curve (AUC_{iv}), terminal half-life ($t_{1/2}$), total systemic clearance (CL) and steady state volume of distribution (V_{ss}) were obtained after i.v. application of labelled R/S-verapamil. After oral administration of unlabelled R/S-verapamil peak serum concentration (C_{max}) and time of C_{max} (t_{max}) were obtained from the serum concentration-time curve. AUC_{po} of unlabelled R/S-verapamil was calculated by the trapezoidal rule over the dosing interval (1–25 h), apparent oral clearance (CL_o) was derived from the equation $CL_o = (\text{dose}_{po} / AUC_{po})$ and absolute bioavailability of R/S-verapamil 240 mg ($F_{measured}$) was obtained from $F_{measured} = (AUC_{po} * \text{dose}_{iv}) / (AUC_{iv} * \text{dose}_{po}) * 100$. Prediction of bioavailability ($F_{predicted}$) was deduced from $1/F = (CL_o / Q_H) + 1$ with liver blood flow being $Q_H = (25.3 \text{ ml min}^{-1} \text{ kg}^{-1}) * \text{body weight}$ [12, 13]. Serum samples for enantioselective analysis were chosen at $t_{min,po}$ and $t_{max,po}$ of each subject following morning and evening treatment in order to calculate R- to S-verapamil serum concentration ratios. Besides, d₇-R- to d₇-S-verapamil serum concentration ratios were determined at t_{1h} ($= t_{min,po}$).

Pharmacodynamic analysis

For any given time point the percentage change in ECG derived PR interval was calculated by subtracting the baseline value (morning *vs* evening) measured just prior to the start of a treatment phase from PR interval measured during the corresponding study day.

Statistical analysis

Data are presented as means with 95% confidence intervals. Effects of dosing time, sequence, subject (within

sequence) and period on pharmacokinetic and pharmacodynamic parameters were tested by multifactor ANOVA (STATGRAPHICS Plus®). Characteristics after i.v. and p.o. administration were evaluated in separate ANOVAs. *Post hoc* we analysed the data of sex differences. In analogy with bioequivalence studies 90% confidence intervals of the various log-transformed parameters after evening treatment relative to morning treatment were derived from the residual variance in ANOVA. Equivalence between morning and evening treatment was concluded if the 90% confidence intervals of the differences (AUC_{po}, C_{max} , AUC_{iv}) were within the equivalence range of 0.8–1.25. Differences in pharmacodynamic baseline values between morning and evening treatment were analysed by Wilcoxon signed rank test, differences in R- to S-verapamil serum concentration ratios by repeated measures ANOVA and correlation of clearance and bioavailability by Spearman rank correlation (GraphPad InStat®). The level of significance was set at $\alpha = 0.05$.

Results

Pharmacokinetic results

Mean serum R/S-verapamil and d₇-R/S-verapamil concentration-time profiles are given in Figure 1. We observed no significant differences in the pharmacokinetic parameters AUC_{po}, C_{max} , t_{max} , CL_o, $F_{measured}$ and R- to S-verapamil serum concentration ratios at $t_{min,po}$ and $t_{max,po}$ between administration of a new R/S-verapamil 240 mg modified release formulation once daily at 08.00 h and 20.00 h (Table 1). There were no statistically significant differences in AUC_{iv}, CL, V_{ss} and d₇-R- to d₇-S-verapamil serum concentration ratios at t_{1h} between morning and evening treatment with a single infusion of

Figure 1 Mean serum concentration-time profiles for d₇-R/S-verapamil (triangle) and R/S-verapamil (circle) after a single dose of 10 mg d₇-R/S-verapamil i.v. 1 h before administration of a 240 mg modified release formulation of R/S-verapamil at steady state to 24 healthy volunteers; morning administration (○) *vs* evening administration (●).

	Morning treatment	Evening treatment	Difference
AUC _{po} (ng ml ⁻¹ h)	1931 (1617, 2245)	1869 (1611, 2128)	62 (−167, 291)
C _{max} (ng ml ⁻¹)	217 (166, 269)	202 (165, 240)	15 (−39, 69)
t _{max} (h)	5.7 (4.9, 6.5)	5.3 (4.4, 6.2)	0.4 (−0.6, 1.4)
CL _o (ml min ⁻¹)	2263 (1773, 2752)	2249 (1846, 2651)	14 (−275, 303)
F _{measured}	0.44 (0.39, 0.50)	0.46 (0.40, 0.51)	−0.02 (−0.08, 0.05)
F _{predicted}	0.65 (0.62, 0.68)	0.64 (0.62, 0.67)	0.01 (−0.01, 0.03)
R/S ratio at t _{min,po}	4.4 (4.2, 4.5)	4.6 (4.4, 4.8)	−0.2 (−0.5, 0)
R/S ratio at t _{max,po}	4.6 (4.3, 5.0)	5.1 (4.8, 5.5)	−0.5 (−0.9, −0.2)

Table 1 Pharmacokinetic parameters after administration of a 240 mg modified release formulation of unlabelled R/S-verapamil to 24 healthy volunteers (steady state dosing; morning *vs* evening treatment). Values are given as mean (95% confidence interval).

	Morning treatment	Evening treatment	Difference
AUC _{iv} (ng ml ⁻¹ h)	182 (162, 202)	170 (156, 183)	13 (−2, 27)
CL (ml min ⁻¹)	896 (798, 994)	933 (863, 1004)	−37 (−112, 37)
V _{ss} (l)	439 (380, 498)	452 (406, 499)	−13 (−74, 48)
t _{1/2} (h)	10.6 (9.0, 12.2)	8.8 (8.1, 9.5)	1.8* (0.3, 3.2)
d ₇ -R/d ₇ -S ratio at t _{1h}	1.9 (1.8, 1.9)	1.8 (1.7, 1.9)	0.1 (0, 0.1)

Table 2 Pharmacokinetic parameters after administration of 10 mg d₇-R/S-verapamil i.v. to 24 healthy volunteers (single dose, morning *vs* evening treatment). Values are given as mean (95% confidence interval).

**P* = 0.0485 morning treatment *vs* evening treatment.

Figure 2 No correlation between total systemic clearance and measured bioavailability of verapamil (circle); $F_{\text{measured}} = (\text{AUC}_{\text{po}} \times \text{dose}_{\text{iv}}) / (\text{AUC}_{\text{iv}} \times \text{dose}_{\text{po}}) \times 100$; morning administration (○, $r = -0.13$, NS) *vs* evening administration (●, $r = -0.12$, NS). Correlation between total systemic clearance and predicted bioavailability of verapamil (cross); $F_{\text{predicted}}$ was deduced from $1/F = (\text{CL}_o/Q_H) + 1$ with $Q_H = (25.3 \text{ ml min}^{-1} \text{ kg}^{-1}) \times \text{body weight}$; morning administration (+, $r = -0.80$, $P < 0.0001$) *vs* evening administration (+, $r = -0.88$, $P < 0.0001$).

10 mg d₇-R/S-verapamil, but $t_{1/2}$ was slightly longer after morning dosing, 10.6 *vs* 8.8 h (Table 2). We found no correlation between CL and F_{measured} (Figure 2). Equivalence between morning and evening treatment was concluded as for the confirming criteria AUC_{po}, C_{max} and AUC_{iv} the respective point estimate and 90% confidence interval were within the equivalence range (Table 3). In addition, stratification by sex is reported with the parameters AUC_{po}, C_{max}, AUC_{iv}, CL and V_{ss} being corrected for body-weight (Table 4). Female gender

Table 3 Summary of equivalence assessment between morning treatment and evening treatment in 24 healthy volunteers. AUC_{iv} is the confirming criterion for concluding equivalence of morning and evening infusion. CL, V_{ss} and $t_{1/2}$ were evaluated exploratively.

	Point estimate	90% confidence interval
(a) 240 mg modified release formulation of unlabelled R/S-verapamil (steady state)		
AUC _{po} (ng ml ⁻¹ h)	0.99	0.89–1.09
C _{max} (ng ml ⁻¹)	0.98	0.80–1.19
(b) 10 mg d ₇ -R/S-verapamil i.v. (single dose)		
AUC _{iv} (ng ml ⁻¹ h)	0.95	0.89–1.01
CL (ml min ⁻¹)	1.06	0.99–1.13
V _{ss} (l)	1.05	0.95–1.15
t _{1/2} (h)	0.87	0.79–0.96

was associated with higher CL and V_{ss} during both times of i.v. dosing.

Pharmacodynamic results

Baseline values of PR interval measured in the morning and in the evening prior to the start of each treatment phase were not significantly different (156; 150–162 ms *vs* 160; 153–166 ms). During each study day (0–25 h) mean changes in PR interval from baseline (%) were similar for either time of administration as the AUEC_{PR} (0, 25 h) of both response curves did not differ significantly (221; 172–270%PR h *vs* 169; 100–238%PR h; Figure 3). With the response curves being divided into an 'i.v. segment' AUEC_{PR} (0, 1 h) and an 'oral segment' AUEC_{PR} (1, 25 h) we found a significantly

Table 4 Stratification by sex (12 healthy females and 12 healthy males). Values are given as mean (95% confidence interval).

	Morning treatment		Evening treatment	
	Females	Males	Females	Males
(a) 240 mg modified release formulation of unlabelled R/S-verapamil (steady state)				
AUC _{po} (ng ml ⁻¹ h kg ⁻¹)	30 (22, 39)	29 (21, 36)	28 (21, 36)	29 (24, 34)
C _{max} (ng ml ⁻¹ kg ⁻¹)	3.8 (2.0, 5.5)	3.0 (2.0, 4.1)	3.0 (2.0, 4.0)	3.1 (2.5, 3.8)
t _{max} (h)	5.4 (4.7, 6.1)	6.0 (4.3, 7.7)	5.7 (4.8, 6.5)	5.0 (3.3, 6.7)
F _{measured}	0.45 (0.35, 0.55)	0.44 (0.37, 0.51)	0.44 (0.35, 0.52)	0.48 (0.41, 0.56)
AUEC _{PR,1-25 h} (%PR h)	220 (138, 302)	178 (119, 236)	153 (90, 215)	158 (32, 284)
(b) 10 mg d ₇ -R/S-verapamil i.v. (single dose)				
AUC _{iv} (ng ml ⁻¹ h kg ⁻¹)	2.8 (2.3, 3.3)	2.7 (2.2, 3.2)	2.6 (2.3, 3.0)	2.5 (2.2, 2.8)
CL (ml min ⁻¹ kg ⁻¹)	17* (13, 20)	11 (10, 13)	17* (15, 19)	12 (11, 13)
V _{ss} (l kg ⁻¹)	8.4* (6.3, 10.4)	5.4 (4.6, 6.1)	8.2* (7.0, 9.4)	5.8 (4.8, 6.8)
t _{1/2} (h)	10.6 (9.1, 12.1)	10.6 (7.4, 13.8)	9.1 (7.9, 10.3)	8.5 (7.7, 9.4)
AUEC _{PR} (0, 1 h) (%PR h)	23 (19, 27)	22 (15, 28)	15 (9, 22)	13 (3, 23)

P* < 0.001 females *vs* males. Figure 3** Time course of mean increase in PR interval from baseline; morning administration (○) *vs* evening administration (●) of 10 mg d₇-R/S-verapamil i.v. (single dose) at t_{0h} and a 240 mg modified release formulation of R/S-verapamil (steady state) at t_{1h}.

greater PR prolongation after verapamil i.v. in the morning than in the evening (22; 19–26%PR h *vs* 14; 8–20%PR h; *P* = 0.0128), but no difference between mean PR increase after verapamil p.o. at 08.00 h and 20.00 h, respectively (199; 152–246%PR h *vs* 155; 91–220%PR h). Time to maximum PR prolongation differed not significantly between morning and evening dosing of verapamil p.o. (6.6; 5.7–7.6 h *vs* 6.0; 5.0–6.9 h). Ratios of AUEC_{PR} (0, 1 h) to AUC_{iv} (0, 1 h) reflecting increase in PR interval per unit of serum drug concentration were not different between morning and evening treatment (0.52; 0.37–0.67%PR ml ng⁻¹ *vs* 0.38; 0.23–0.53%PR ml ng⁻¹). In parallel ratios of AUEC_{PR} (1, 25 h) to AUC_{po} (1, 25 h) did not differ between morning and

evening administration (0.11; 0.08–0.15%PR ml ng⁻¹ *vs* 0.09; 0.05–0.14%PR ml ng⁻¹).

Discussion

No significant influence of time of dosing was found on pharmacokinetics and prolongation of PR interval following a new R/S-verapamil 240 mg modified release formulation under steady state conditions in 24 fed healthy volunteers. Absolute bioavailability and systemic clearance has been assessed by simultaneous infusion of deuterated verapamil and was not affected by the time of drug administration.

Following morning infusion of a single dose of 10 mg verapamil we observed a clinically irrelevant, slightly longer elimination half-life and more pronounced effect on atrioventricular conduction than following evening infusion. The reason for this variation is not clear. The ratio of the area under the response curve to the area under the concentration–time curve did not show circadian variation. Since prolongation of the atrioventricular conduction is mainly attributable to the S-enantiomer of racemic verapamil [14] we studied possible temporal changes in enantioselective metabolism of verapamil. However, d₇-R- to d₇-S-verapamil serum concentration ratios were not different between morning and evening infusion. Possible changes in protein binding of verapamil did not result in significant circadian changes of volume of distribution.

Clinical studies on chronopharmacokinetics and chronopharmacodynamics of verapamil are rare and controversial. Comparability is limited since certain variables influencing pharmacokinetics often differ and sometimes they are not kept constant. In particular, protocols differ

in galenic formulations used, fasting times, meals, posture and activity of subjects or patients and multiple or single doses of verapamil. In none of these studies verapamil was given intravenously. To see whether circadian variation in presystemic metabolism of verapamil occurs we employed stable isotope technique which allows simultaneous and differential measuring of orally and intravenously administered drugs and excludes intraindividual variability in drug disposition. We conducted the study under usual life conditions with a lighter meal at breakfast than at supper as differences of meal condition in our daily life may play a role in the mechanism underlying circadian changes of drug absorption [15].

An early small study on chronopharmacology of sustained release verapamil revealed a significant influence of time of dosing in eight hypertensive patients [16]. Verapamil $C_{\max}$ and AUC values were reduced when once-daily controlled release tablets were administered at 20.00 h as compared with administration at 08.00 h. A few years later the same investigators designed another study in eight healthy volunteers to clarify the effect of time of administration (04.00 h, 08.00 h, 12.00 h, 16.00 h, 20.00 h, 24.00 h) on pharmacokinetics after a single oral dose of verapamil [17]. Differences were higher peak concentrations and bioavailabilities after the 08.00 h and noon administration than at any other time. A multiple-dose study in 24 healthy volunteers found that verapamil $C_{\max}$ and AUC values decreased significantly, if immediate release tablets of verapamil were administered at 16.00 h and midnight relative to the corresponding values from 08.00 h [18]. The investigators attributed their observation to reduced drug absorption during evening and night. However, food effects cannot be ruled out as timing of meals and fasting intervals before ingestion of verapamil had not been strictly controlled. These findings are in contradiction to another multiple-dose study in 29 fasting subjects [19]. After morning administration of an osmotically controlled formulation of verapamil $C_{\max}$ and $t_{\max}$ values were significantly lower compared with values observed after night administration. Mean AUC values were not different. The authors concluded that time of dosing had a slight effect on the rate of absorption but no effect on the extent of absorption.

Absorption of modified release verapamil given in our study was not affected by time of dosing. Higher and earlier peak serum concentration without altered AUC within a dosing interval of verapamil was not found in one of the two treatment periods of this strictly controlled chronopharmacological study. Circadian change in hepatic blood flow altering hepatic extraction can be ruled out as circadian influence on pharmacokinetics of this new formulation of modified release verapamil as systemic clearance remained unchanged at different times of dosing.

So called predicted bioavailability of verapamil which

is based upon oral clearance data would not have been remarkably higher than bioavailability of verapamil actually measured if this new formulation of verapamil had been fully absorbed from the gastrointestinal tract without any prehepatic metabolism. In parallel there was no correlation between absolute bioavailability and systemic clearance indicating substantial prehepatic metabolism probably due to retardation of intestinal drug release.

Only one of the quoted studies investigated the influence of time of dosing on atrioventricular conduction [19] in addition to chronopharmacokinetics of verapamil. Mean changes in PR interval following morning and evening treatment with a retarded formulation of verapamil were described as almost superimposable. Our investigation supports the absence of chronopharmacodynamic effects in modified release verapamil, but PR-prolongation was significantly greater after verapamil i.v. in the morning than in the evening.

As women are underrepresented in clinical trials [20] we included subjects of both sexes into the study. Japanese investigators detected no gender-related differences in the pharmacokinetics of oral verapamil [21]. However, mean plasma verapamil concentrations of both enantiomers were higher for women than for men at all time points following administration of an oral sustained release formulation of verapamil [22]. Female gender was associated with higher *S*-verapamil clearances after single i.v. doses of *S*-verapamil and during multistage racemic verapamil infusions [23]. We also found a gender effect on systemic clearance of verapamil after single dose infusion with higher systemic clearances and higher steady state volumes of distribution in females than in males, but we detected no other gender-related differences in pharmacokinetics and pharmacodynamics of i.v. and oral modified release verapamil. The small subgroup sizes in our study do not allow a definite analysis of gender-related effects.

In summary, we demonstrated that time of dosing has no significant influence on pharmacokinetics and pharmacodynamics of this new modified release formulation of verapamil. Circadian variation in presystemic metabolism of verapamil was not observed.

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