Population analysis of the non linear red blood cell partitioning and the concentration-effect relationship of draflazine following various infusion rates

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Aims To investigate the impact of the specific red blood cell binding on the pharmacokinetics and pharmacodynamics of the nucleoside transport inhibitor draflazine after i.v. administration at various infusion rates. It was also aimed to relate the red blood cell (RBC) occupancy of draflazine to the *ex vivo* measured adenosine breakdown inhibition (ABI).

Methods Draflazine was administered to healthy volunteers as a 15-min i.v. infusion of 0.25, 0.5, 1, 1.5 and 2.5 mg immediately followed by an infusion of the same dose over 1 h. Plasma and whole blood concentrations were measured up to 120 h post dose, and were related to the *ex vivo* measured ABI, serving as a pharmacodynamic endpoint. The capacity-limited specific binding of draflazine to the nucleoside transporter located on the erythrocytes was evaluated by a population approach.

Results The estimate of the population parameter typical value (%CV) of the binding constant K_d and the maximal specific binding capacity (B_{max}) was 0.385 (3.5) ng ml^{-1} plasma and 158 (2.1) ng ml^{-1} RBC, respectively. The non-specific binding was low. The specific binding to the erythrocytes was a source of non-linearity in the pharmacokinetics of draflazine. The total plasma clearance of draflazine slightly decreased with increasing doses, whereas the total clearance in whole blood increased with increasing doses. The sigmoidal E_{max} equation was used to relate the plasma and whole blood concentration of draflazine to the *ex vivo* determined ABI. In plasma, typical values (%CV) of E_{max}, IC_{50} and Hill factor were 81.4 (1.9)%, 3.76 (9.3) ng ml^{-1} and 1.06 (3.4), respectively. The relationship in whole blood was much steeper with population parameter typical values (%CV) of E_{max}, IC_{50} and Hill factor of 88.2 (2.0)%, 65.7 (2.8) ng ml^{-1} and 4.47 (5.5), respectively. The RBC occupancy of draflazine did not coincide with the *ex vivo* measured ABI. The observed relationship between RBC occupancy and ABI was not directly proportional but similar for all studied infusion schemes.

Conclusions The findings of this study show that the occupancy of the nucleoside transporter by draflazine should be at least 90% in order to inhibit substantially adenosine breakdown *in vivo*. On the basis of these findings it is suggested that a 15 min infusion of 1 mg draflazine followed by an infusion of 1 mg h^{-1} could be appropriate in patients undergoing a coronary artery bypass grafting.

Keywords: draflazine, nucleoside transport inhibitor, population analysis, non-linear red blood cell partitioning, pharmacokinetics, pharmacodynamics, adenosine breakdown inhibition, red blood cell occupancy

Introduction

Endogenous adenosine is released in the intercellular interstitial space of cardiac tissue in response to any negative balance between energy supply and energy demand [1, 2]. The multitude of pharmacological properties of the nucleoside adenosine may limit myocardial damage from ischaemia and reperfusion [3–6]. However, the released adenosine is rapidly catabolised, so that the cardioprotective effect is fairly transient [7–8].

An important role in the fate of adenosine is played by the nucleoside transporter which facilitates the intracellular uptake of adenosine. Nucleoside transporters are located on the endothelial cells lining the vasculature as well as on red blood cells [9, 10]. Inhibition of the nucleoside transporter will prevent the intracellular uptake and subsequent catabolism of adenosine into inosine and hypoxanthine.

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Consequently, the presence of extracellular adenosine will be prolonged so that it may reach concentrations sufficiently high to exert cardioprotective effects.

Draflazine is a nucleoside transport inhibitor with cardioprotective effects in various *in vitro* and *in vivo* models for cardioprotection [11–17]. One of the possible indications for draflazine is cardiac protection during cardiac surgery such as coronary artery bypass grafting (CABG). In the first-time-to-man study of draflazine, a 15 min i.v. infusion of 2.5 mg draflazine was administered to healthy male subjects [18]. This study showed that the red blood cell/plasma distribution of draflazine was concentration dependent and could be characterized on the basis of a specific binding to the nucleoside transporter on the red blood cells [19, 20]. This capacity-limited specific binding appeared to be a source of non-linearity in the pharmacokinetics of draflazine. Therefore, the present study in healthy subjects was conducted to investigate further the impact of the specific red blood cell binding on the pharmacokinetics and pharmacodynamics of draflazine after i.v. administration at various infusion rates. The population average specific binding parameters of draflazine and the inter- and intraindividual variability of this red blood cell binding were determined by a non-linear mixed effect method (NONMEM). In addition, the population pharmacokinetic-dynamic parameters of the ABI–draflazine concentration relationship as well as the inter- and intraindividual variability of these parameters were also determined by a population approach (NONMEM).

In a previous study it was suggested that the red blood cell nucleoside transporter occupancy of draflazine (RBC occupancy) could serve as a useful pharmacodynamic endpoint for future dose ranging studies [18]. Therefore, in the present study, the RBC occupancy of draflazine was evaluated for various infusion rates of draflazine and was related to the *ex vivo* measured adenosine breakdown inhibition (ABI).

Methods

Subjects

The subjects participated in this study after having given their voluntary written informed consent. All subjects were male Caucasians between the ages of 18 and 55 years with a body weight within 20% of the ideal weight as described in the Metropolitan Life Insurance Company's Height and Weight Table. All were in good health as established by medical history, physical examination, ECG and results of blood biochemistry, haematology and urinalysis testing within 2 weeks prior to the start of the study. The subject characteristics are given in Table 1.

The use of all medication, with the exception of paracetamol, had to be stopped at least 14 days prior to the start of the study. Paracetamol was permitted up to 3 days before the start of each study session.

Study design

The study was conducted according to the principles of the Declaration of Helsinki and its subsequent revisions. The approval of the study was obtained from a recognised Ethics Committee.

This randomized double-blind placebo-controlled dose-escalation study consisted of separate study sessions. Before the start of each study session, subjects had fasted overnight and the intake of alcoholic beverages, coffee, tea, cola, chocolate or any other product containing caffeine or cocoa was forbidden from 24 h before till the end of each session.

In five study sessions, two alternating groups of 12 subjects received a 15 min i.v. infusion of 0.25, 0.5, 1, 1.5 and 2.5 mg draflazine immediately followed by the same dose over 1 h. The i.v. solutions of draflazine or placebo were infused in a forearm vein by means of an infusion pump. Subjects remained in supine position from the start of the intravenous infusion until 60 min after the end of the 1 h infusion.

Within each study session, eight subjects received an infusion of draflazine and the other four subjects received placebo. The clinical investigator was only aware of the administered dose of draflazine in each session.

Venous blood was collected from an arm vein immediately prior to and at specific time points after the start of the infusion up to 120 h post-infusion. Per subject, a total number of 15 blood samples were taken in each study session. Blood samples during the first day were taken from the arm opposite to the infusion arm. A 7.5 ml blood sample was divided over two heparinized tubes. One 2.5 ml blood sample was stored at -20° C until assayed for draflazine. Plasma from the other 5 ml blood sample was harvested and was stored at -20° C until the time of drug assay. At the times of blood sampling for the determination of the drug concentration, additional 4 ml blood samples were collected on acid citrate dextrose (ACD) for the *ex vivo* determination of the adenosine breakdown inhibition. These samples were stored at 4° C and were kept on ice during the weekly transport to the site of analysis. Previous data have shown

Table 1 Subject characteristics.

Infusion scheme	*Age median (min–max) (years)*	*Body weight median (min–max) (kg)*	*Height median (min–max) (cm)*
0.25 mg + 0.25 mg h^{-1} for 1 h	23.0 (19–48)	79.0 (62–108)	183.5 (177–198)
0.5 mg + 0.5 mg h^{-1} for 1 h	22.0 (20–26)	81.5 (64–93)	182.0 (173–191)
1 mg + 1 mg h^{-1} for 1 h	22.5 (20–48)	78.5 (59–87)	181.0 (175–188)
1.5 mg + 1.5 mg h^{-1} for 1 h	21.5 (19–26)	74.0 (70–87)	181.0 (177–186)
2.5 mg + 2.5 mg h^{-1} for 1 h	22.5 (20–23)	74.0 (64–85)	180.0 (172–191)

that no change in the *ex vivo* breakdown of adenosine was observed up to a storage period of 12 days at 4° C.

Drug concentrations

Concentrations of draflazine in whole blood were determined by reversed phase high-performance liquid chromatography with u.v. detection at 254 nm [21]. The limit of quantification was 5 ng ml^{-1}. The accuracy (RE) and precision (CV) obtained from independently prepared quality control samples was respectively +6.6% and 6.5% at 16.2 ng ml^{-1} (n=22), +1.1% and 3.8% at 101 ng ml^{-1} (n=21) and −1.4% and 4.5% at 486 ng ml^{-1} (n=21).

Plasma concentrations of draflazine were determined by a red blood cell binding assay after selective extraction [22]. The limit of quantification was 0.10 ng ml^{-1} plasma. The RE and CV obtained from independently prepared quality control samples was respectively −3.0% and 15.8% at 0.114 ng ml^{-1} (n=6), +6.2% and 9.7% at 0.510 ng ml^{-1} (n=6) and −1.8% and 9.9% at 3.32 ng ml^{-1} (n=6).

Ex vivo *assessment of adenosine breakdown inhibition (ABI)*

Blood samples on ACD were centrifuged and an erythrocyte suspension was made. Then, adenosine was added to a final concentration of 40 μM. Samples were centrifuged after being incubated for 20 min at 25° C. The pellet was discarded and the final clear supernatant was stored at −20° C until assayed for adenosine, inosine and hypoxanthine. This method was described in more detail by Wainwright *et al.* [23] and Van Belle *et al.* [14].

Total concentrations of adenosine, inosine and hypoxanthine in the final supernatant were measured using h.p.l.c. as described previously [24, 25]. Then, the adenosine concentration (f_{ADO}) was calculated as the fraction of the sum of the concentrations of adenosine, hypoxanthine and inosine. The adenosine breakdown inhibition (ABI) was expressed as a percentage and was calculated as:

$$ABI_{ij}\ (\%) = \frac{f_{ADO_{ij}} - f_{ADO_{0j}}}{1 - f_{ADO_{0j}}} \cdot 100 \qquad (1)$$

where ABI_{ij} is the percentage inhibition of the breakdown of adenosine in sample i of subject j. $f_{ADO_{0j}}$ and $f_{ADO_{ij}}$ represent the fraction of adenosine in the *jth* individual as determined in the sample taken just before and in the *ith* sample after the start of the draflazine infusion, respectively.

Population RBC/plasma distribution model

For each individual subject, the total red blood cell concentration of draflazine was calculated from the measured whole blood and plasma concentration of draflazine as:

$$C_{RBC_{ij}} = \frac{C_{b_{ij}} - (1 - H_j) \cdot C_{P_{ij}}}{H_j} \qquad (2)$$

where $C_{b_{ij}}$ and $C_{p_{ij}}$ are respectively the whole blood concentration and the plasma concentration of draflazine measured in sample i of individual j and H_j is the measured haematocrit of the jth subject. $C_{RBC_{ij}}$ is the calculated total red blood cell concentration of draflazine in the ith sample of subject j. $C_{RBC_{ij}}$ served as a dependent variable of the population RBC/plasma distribution model, and was used for the subsequent estimation of the erythrocyte binding parameters (equation 3).

As draflazine exhibits a capacity-limited high-affinity binding to the transporters located on the red blood cells [18, 20], the total red blood cell concentration of draflazine was related to the plasma concentration as:

$$\hat{C}_{RBC_{ij}} = \frac{\hat{B}_{max_j} \cdot C_{p_{ij}}}{\hat{K}_{d_j} + C_{p_{ij}}} + \hat{K}_{non\text{-}specific_j} \cdot C_{p_{ij}} \qquad (3)$$

where $\hat{C}_{RBC_{ij}}$ is the model-predicted ith total red blood cell concentration of draflazine in the jth individual at the measured plasma concentration $C_{p_{ij}}$. The measured $C_{p_{ij}}$ served as the independent variable of the population RBC/plasma model. $\hat{B}_{max_j}$ is the Bayesian estimate of the maximal concentration of draflazine specifically bound to erythrocytes in the jth individual. $\hat{K}_{d_j}$ is the predicted dissociation constant in subject j, defined as the draflazine plasma concentration at which 50% of $\hat{B}_{max_j}$ is specifically bound to the red blood cells. Finally, $\hat{K}_{non\text{-}specific_j}$ is the Bayesian estimate of the constant expressing the non-specific erythrocyte binding in the jth volunteer.

Based on the Bayesian estimates of the specific binding parameters in each individual subject, the percentage red blood cell nucleoside transporters occupied by draflazine (RBC occupancy$_{ij}$) was calculated for each measured plasma concentration ($C_{p_{ij}}$) as:

$$\text{RBC occupancy}_{ij}\ (\%) = \frac{C_{RBC,specific_{ij}}}{\hat{B}_{max\ j}} \cdot 100 = \frac{C_{p_{ij}}}{\hat{K}_{d_j} + C_{p_{ij}}} \cdot 100 \qquad (4)$$

Pharmacokinetic analysis

The capacity-limited binding of draflazine to the red blood cells appeared to be a source of non linearity in the pharmacokinetics of the drug in plasma as well as in whole blood [18]. Therefore, a compartmental model could not be used to describe the plasma and blood concentration-time profiles of draflazine after different infusion schemes.

Non-compartmental pharmacokinetic parameters were determined based on the individual plasma and blood concentration-time curves using the actual sampling times. The initial half-life post-infusion ($t_{1/2,post\text{-}infusion}$) and the terminal half-life ($t_{1/2,z}$) were determined by linear regression of the post-infusion and terminal points of the log-linear concentration-time curves, respectively. The total plasma and blood clearance of draflazine was calculated by dividing the infused dose by the total AUC.

Population concentration-effect relationship model

The sigmoidal E_{max} or Hill equation was used to relate the measured plasma and whole blood concentrations of each individual subject to the *ex vivo* measured adenosine

breakdown inhibition:

$$\hat{ABI}_{ij}(\%) = \frac{\hat{E}_{max_j} \cdot C_{ij}^{\hat{\gamma}_j}}{\hat{IC}_{50_j}^{\hat{\gamma}_j} + C_{ij}^{\hat{\gamma}_j}} \quad (5)$$

where $\hat{ABI}_{ij}$ is the model-predicted ith percentage adenosine breakdown inhibition in individual j at the measured concentration C_{ij}. The measured plasma or whole blood concentration of draflazine (C_{ij}) served as the independent variable of the population concentration-effect relationship model. $\hat{E}_{max_j}$ is the Bayesian estimate of the maximal percentage adenosine breakdown inhibition in the jth subject. The parameter $\hat{IC}_{50_j}$ represents the predicted concentration of draflazine in the jth individual that produces 50% of the maximal adenosine breakdown inhibition; and $\hat{\gamma}_j$ is the Bayesian estimate of the factor describing the steepness of the sigmoidal E_{max} relationship in the subject j.

Separate independent sigmoidal E_{max} population models were constructed for the measured ABI related to the measured plasma concentration of draflazine and for the ABI related to the whole blood concentration of draflazine.

Statistical models

RBC/plasma distribution model. A log-normal distribution was assumed to describe the interindividual variability of the parameters. To express the log-normal distribution in the regression models, log-additive random interindividual error was assumed. Thus for the parameters B_{max}, K_d and $K_{non\text{-}specific}$ we can write:

$$\ln B_{max_j} = \ln \hat{B}_{max} + \eta_{ij}^{B_{max}} \quad (6)$$

$$\ln K_{d_j} = \ln \hat{K}_d + \eta_{ij}^{K_d} \quad (7)$$

$$\ln K_{non\text{-}specific_j} = \ln \hat{K}_{non\text{-}specific} + \eta_{ij}^{K_{non\text{-}specific}} \quad (8)$$

where B_{max_j}, K_{d_j} and $K_{non\text{-}specific_j}$ are the true parameters in subject j and $\hat{B}_{max}$, $\hat{K}_d$ and $\hat{K}_{non\text{-}specific}$ represent the typical values for the population. $\eta_{ij}^{B_{max}}$, $\eta_{ij}^{K_d}$ and $\eta_{ij}^{K_{non\text{-}specific}}$ are random variables with a mean value zero and variance $\omega^2_{B_{max}}$, $\omega^2_{K_d}$ and $\omega^2_{K_{non\text{-}specific}}$, respectively.

The following general model with a proportional and an additional error term was assumed for the remaining unexplained *intra*individual variability:

$$C_{RBC_{ij}} = \hat{C}_{RBC_{ij}} + \sqrt{\theta^2_{RBC_1} \cdot \hat{C}^{\,2}_{RBC_{ij}} + \theta^2_{RBC_2}} \cdot \varepsilon_{RBC_{ij}} \quad (9)$$

where $C_{RBC_{ij}}$ is the ith total red blood cell concentration in the jth individual, and $\hat{C}_{RBC_{ij}}$ is the corresponding predicted concentration from equation 3. The variables θ_{RBC_1} and θ_{RBC_2} are proportional and additional error terms, respectively. Finally, $\varepsilon_{RBC_{ij}}$ denotes the residual intrasubject random error distributed normally with zero mean and unit variance.

Concentration-effect relationship model. A log-additive random inter-individual error was assumed for the description of the interindividual variability of the parameters E_{max} and IC_{50}:

$$\ln E_{max_j} = \ln \hat{E}_{max} + \eta_{ij}^{E_{max}} \quad (10)$$

$$\ln IC_{50_j} = \ln \hat{IC}_{50} + \eta_{ij}^{IC_{50}} \quad (11)$$

where E_{max_j} and IC_{50_j} are the true parameters in subject j. $\hat{E}_{max}$ and $\hat{IC}_{50}$ represent the typical values for the population. $\eta_{ij}^{E_{max}}$ and $\eta_{ij}^{IC_{50}}$ are random variables with a mean value zero and variance $\omega^2_{E_{max}}$ and $\omega^2_{IC_{50}}$, respectively.

In preliminary analyses, a similar parameter η_{ij}^{γ} was included in the model to account for the interindividual

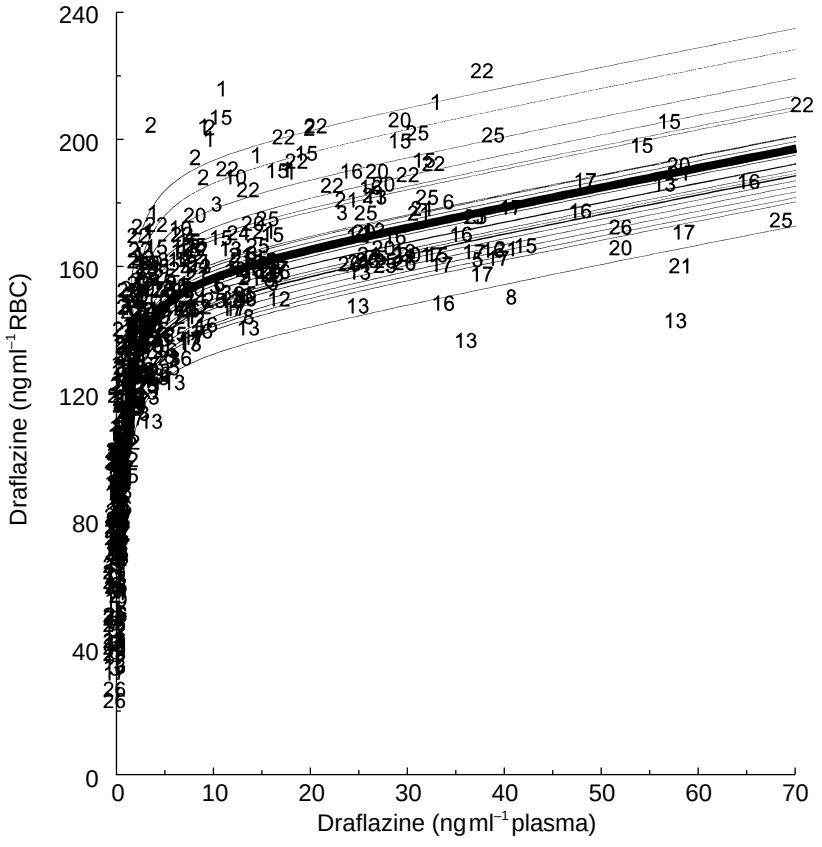

Figure 1 Calculated individual total red blood cell concentrations of draflazine (equation 2) as a function of the plasma concentration of draflazine. The values for each individual subject are indicated by the subject number. The thin lines depict the predicted total red blood cell concentrations of draflazine based on the individual parameter estimates (equation 3). The thick line depicts the predicted total red blood cell concentrations based on the average population parameter estimates.

variability of γ. However, parameter estimation of this highly nonlinear sigmoidal E_{max} model was not feasible with the inclusion of an interindividual variability of this parameter. Therefore, estimation of the interindividual variability in γ was dropped from the final analysis and the interindividual variability in γ was assumed to be zero.

The remaining unexplained *intra*individual variability was described by a general model with an additional as well as a proportional error term:

$$ABI_{ij} = \hat{ABI}_{ij} + \sqrt{\theta^2_{ABI_1} \cdot \hat{ABI}^2_{ij} + \theta^2_{ABI_2}} \cdot \varepsilon_{ABI_{ij}} \qquad (12)$$

where ABI_{ij} is the *i*th adenosine breakdown inhibition in the *j*th individual, and $\hat{ABI}_{ij}$ is the corresponding predicted ABI from equation 5. θ_{ABI_1} and θ_{ABI_2} are proportional and additional error terms, respectively. Finally, $\varepsilon_{ABI_{ij}}$ denotes the residual intrasubject random error distributed with zero mean and a variance τ^2_{ABI} equal to unity.

Computation

Estimation of the population parameters and model building were performed using the program NONMEM [26, 27] version IV level 1.2, which was run on a HP-9000 workstation operating under HP/UX. The model equations were written as abbreviated code in the $PRED block of the NM-TRAN control stream file.

Results

Figure 1 depicts the calculated and predicted red blood cell concentrations of draflazine as a function of the plasma

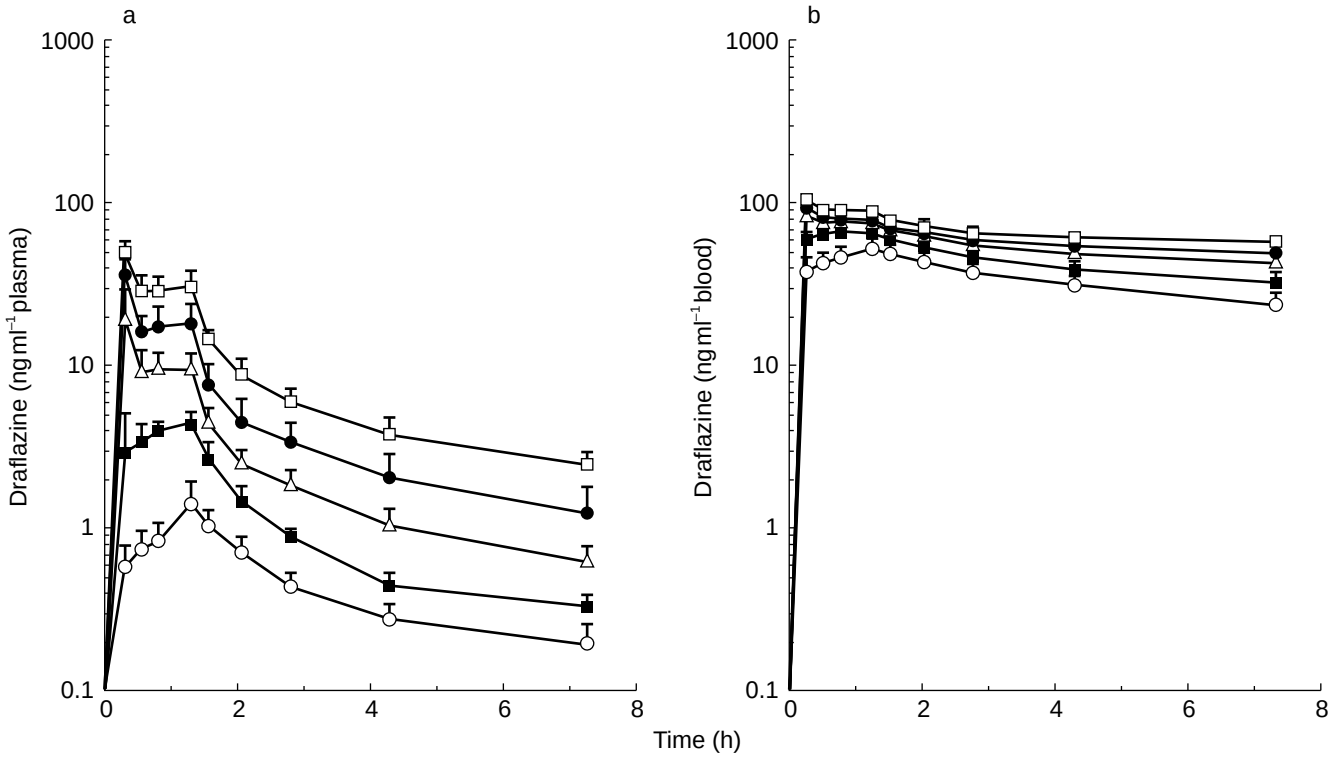

Figure 2 a) Mean (+s.d.) plasma concentrations of draflazine as a function of time after a 15 min i.v. infusion of 0.25 (○), 0.5 (■), 1 (△), 1.5 (●) and 2.5 (□) mg draflazine followed by a 1 h infusion of the same dose in eight healthy male subjects. b) Mean (+s.d.) whole blood concentrations of draflazine as a function of time after a 15 min i.v. infusion of 0.25 (○), 0.5 (■), 1 (△), 1.5 (●) and 2.5 (□) mg draflazine followed by a 1 h infusion of the same dose in eight healthy male subjects.

Table 2 NONMEM parameter estimates of the population red blood cell/plasma distribution model.

				Interindividual variability		*Intraindividual variability*			
						Proportional error		*Additional error*	
Parameter (theta)		*Parameter value*	*SE* (%CV)*	*Omega (%CV)*	*SE (%CV)*	*Value (%CV)*	*SE (%CV)*	*Value (ng ml^{-1} RBC)*	*SE (%CV)*
B_{max}	(ng ml^{-1} RBC)	158	2.1	9.8	3.1	5.5	14.1	7.04	10.6
K_d	(ng ml^{-1} plasma)	0.385	3.5	13.1	8.9				
$K_{non\text{-}specific}$		0.615	8.7	–	–				

*SE: standard error of the estimate as calculated by NONMEM.

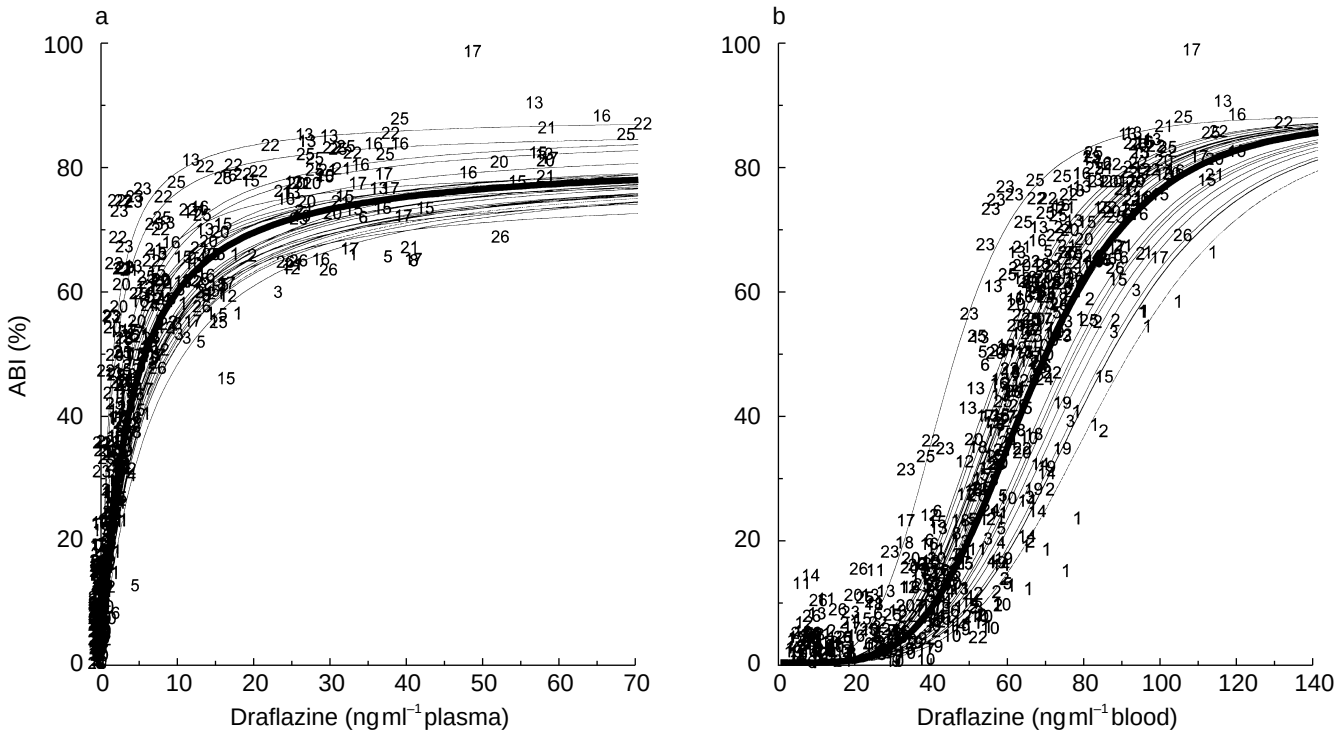

Figure 3 a) Measured individual *ex vivo* adenosine breakdown inhibition (ABI) as a function of the plasma concentrations of draflazine. The values for each individual subject are indicated by the subject number. The thin lines depict the predicted ABI based on the individual parameter estimates (equation 5). The thick line depicts the predicted ABI based on the average population parameter estimates. b) Measured individual *ex vivo* adenosine breakdown inhibition (ABI) as a function of the whole blood concentrations of draflazine. The values for each individual subject are indicated by the subject number. The thin lines depict the predicted ABI based on the individual parameter estimates (equation 5). The thick line depicts the predicted ABI based on the average population parameter estimates.

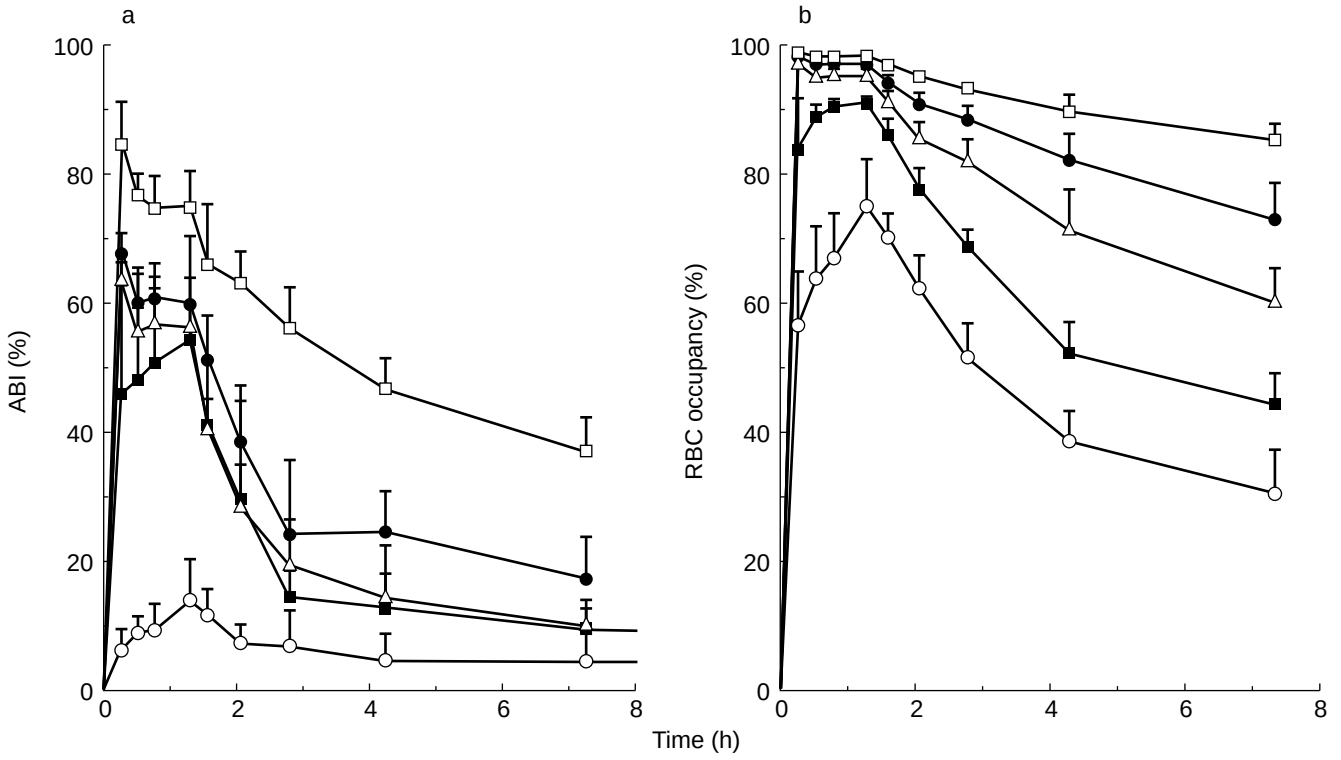

Figure 4 a) Mean (+s.d.) *ex vivo* measured adenosine breakdown inhibition (ABI) as a function of time after a 15 min i.v. infusion of 0.25 (○), 0.5 (■), 1 (△), 1.5 (●) and 2.5 (□) mg draflazine followed by a 1 h infusion of the same dose in eight healthy male subjects. b) Mean (+s.d.) calculated red blood cell occupancy of draflazine as a function of time after a 15 min i.v. infusion of 0.25 (○), 0.5 (■), 1 (△), 1.5 (●) and 2.5 (□) mg draflazine followed by a 1 h infusion of the same dose in eight healthy male subjects.

Table 3 Mean (±s.d.) pharmacokinetic parameters of draflazine in plasma and whole blood after a 15 min i.v. infusion of 0.25, 0.5, 1, 1.5 and 2.5 mg draflazine followed by the same dose as a 1 h infusion in eight healthy male subjects.

	Plasma				*Whole blood*			
Infusion scheme	$C_{end\ 15\text{-}min\ infusion}$ (*ng ml*$^{-1}$)	$C_{end\ 1\text{-}h\ infusion}$ (*ng ml*$^{-1}$)	$t_{1/2,z}$ (*h*)	*CL* (*ml min*$^{-1}$)	$C_{end\ 15\text{-}min\ infusion}$ (*ng ml*$^{-1}$)	$C_{end\ 1\text{-}h\ infusion}$ (*ng ml*$^{-1}$)	$t_{1/2,z}$ (*h*)	*CL* (*ml min*$^{-1}$)
0.25 mg + 0.25 mg h^{-1} for 1 h	0.57 ± 0.20	1.39 ± 0.52	0.9 ± 0.2★	–†	37.3 ± 8.5	52.3 ± 7.5	34.8 ± 14.1	7.2 ± 1.6
0.5 mg + 0.5 mg h^{-1} for 1 h	2.89 ± 2.21	4.27 ± 0.87	0.6 ± 0.1★	–†	60.1 ± 0.6	65.8 ± 5.7	37.8 ± 9.9	9.8 ± 2.8
1 mg + 1 mg h^{-1} for 1 h	19.7 ± 9.5	9.51 ± 2.25	17.9 ± 5.7	902 ± 180	85.2 ± 9.3	75.8 ± 10.7	44.8 ± 9.6	12.2 ± 3.0
1.5 mg + 1.5 mg h^{-1} for 1 h	36.1 ± 8.7	18.3 ± 5.4	20.1 ± 12.2	777 ± 213	94.5 ± 10.5	76.9 ± 7.2	48.0 ± 6.8	15.5 ± 2.3
2.5 mg + 2.5 mg h^{-1} for 1 h	49.8 ± 7.8	30.6 ± 7.4	19.1 ± 4.4	755 ± 137	105 ± 6	88.5 ± 5.0	42.0 ± 3.2	24.0 ± 2.3

★Terminal half-life could not be calculated. Therefore, the post-peak half-life was tabulated. †CL could not be calculated as no terminal half-life could be estimated.

concentration for each individual. Figure 1 also shows the red blood cell concentrations of draflazine calculated for the population typical values of the erythrocyte binding parameters. A visual inspection of Figure 1 revealed that the RBC/plasma distribution of draflazine in each individual subject could be described by the capacity-limited specific binding and non-specific binding of draflazine to the red blood cells. In addition, the variability among subjects was small, which was further demonstrated by NONMEM parameter estimates of the inter- and intraindividual variability (Table 2). The estimate of the population parameter typical value (%CV) of the maximal specific binding capacity B_{max}, the dissociation constant K_d and the non-specific binding constant $K_{non\text{-}specific}$ was 158 (2.1) ng ml^{-1} RBC, 0.385 (3.5) ng ml^{-1} and 0.615 (8.7) respectively. The correlation between the estimated red blood cell binding parameters was negligible.

The mean (±s.d.) concentrations of draflazine in plasma and blood are depicted as a function of time in Figure 2. The mean (±s.d.) pharmacokinetic parameters of draflazine in plasma and blood are listed in Table 3. After cessation of the 1 h infusions, plasma concentrations of draflazine decayed biphasically. Initial post-peak plasma concentrations of draflazine declined very rapidly (Figure 2). As a consequence, plasma concentrations following the two lowest doses of draflazine were only detectable up to 6–24 h post-infusion so that no terminal half-lives could be calculated (Table 3). The mean initial half-life post-infusion ($t_{1/2post\text{-}infusion}$) was 0.6–0.9 h and the terminal half-life ($t_{1/2,z}$) averaged 18–20 h. The total plasma clearance of draflazine decreased with increasing doses (Table 3). After cessation of the 1 h infusions, the biphasic decline of the concentration of draflazine in blood was much slower compared to that in plasma and the mean $t_{1/2,z}$ of draflazine in blood was 35–48 h (Table 3). The total blood clearance of draflazine increased with increasing doses and was much lower compared to that in plasma.

The NONMEM parameter typical values and the parameters expressing the inter- and intra-individual variability of the sigmoidal E_{max} model relating the plasma and blood concentrations of draflazine with the *ex vivo* measured ABI are given in Table 4. The correlation between the parameters of the sigmoidal E_{max} model was negligible. The ABI as a function of the plasma and blood concentration of draflazine for each individual are depicted in Figure 3. Figure 3 also shows the ABI calculated for the population typical values of the parameters of the Hill equation. It is clear from this Figure that the plasma concentration-ABI relationship showed a different profile compared to that in whole blood. The population typical value (%CV) for IC_{50} was about 17 times higher in blood compared with that in plasma, i.e. 3.76 (9.3) ng ml^{-1} plasma and 65.7 (2.8) ng ml^{-1} blood (Table 4). In addition, the sigmoidal E_{max} relationship was much steeper in blood compared to that in plasma as demonstrated by the population typical value (%CV) for the Hill factor γ of 1.06 (3.4) and 4.47 (5.5) in plasma and blood respectively. Table 4 further shows that the interindividual variability in IC_{50} was larger than that for E_{max}.

Means (±s.d.) of the *ex vivo* measured ABI and the calculated RBC occupancy of draflazine are plotted as a

Table 4 NONMEM parameter estimates of the sigmoidal E_{max} model relating the *ex vivo* measured adenosine breakdown inhibition (ABI) to the concentration of draflazine in plasma and whole blood.

				Interindividual variability		*Intraindividual variability*			
						Proportional error		*Additional error*	
Parameter (theta)		*Parameter value*	*SE⋆ (%CV)*	*Omega (%CV)*	*SE (%CV)*	*Value (%CV)*	*SE (%CV)*	*Value (%ABI)*	*SE (%CV)*
Plasma									
E_{max}	(%ABI)	81.4	1.9	5.4	3.9	8.3	24.0	4.5	16.8
IC_{50}	(ng ml^{-1})	3.76	9.3	45.1	14.3				
γ		1.06	3.4	–	–				
Whole blood									
E_{max}	(%ABI)	88.2	2.0	0†	2.8	5.5	99.8	6.4	17.1
IC_{50}	(ng ml^{-1})	65.7	2.8	15.4	4.9				
γ		4.47	5.5	–	–				

⋆SE: standard error of the estimate as calculated by NONMEN. †Interindividual variability was estimated to be very close to zero.

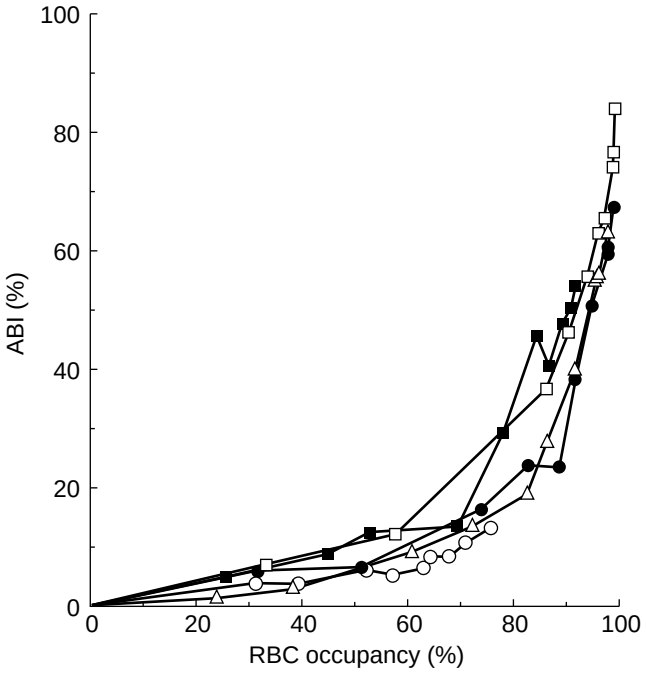

Figure 5 Mean measured *ex vivo* adenosine breakdown inhibition (ABI) as a function of the calculated red blood cell occupancy of draflazine after a 15 min i.v. infusion of 0.25 (○), 0.5 (■), 1 (△), 1.5 (●) and 2.5 (□) mg draflazine followed by a 1 h infusion of the same dose in eight healthy male subjects.

function of time in Figure 4. The ABI-time profiles were different from the RBC occupancy-time profiles. A small ABI was measured during the 15 min infusion of 0.25 mg followed by the same dose over 1 h (Figure 4a). For 1, 1.5 and 2.5 mg, the ABI was maximal at the end of the 15 min infusion and decreased during the subsequent 1 h infusion. The mean ABI at the end of the 15 min infusion of 0.5, 1, 1.5 and 2.5 mg was 45.7%, 63.6%, 67.7% and 84.5%, respectively. At the end of the 1 h infusions of 0.5 mg h^{-1}, 1 mg h^{-1}, 1.5 mg h^{-1} and 2.5 mg h^{-1}, the ABI averaged 54.2%, 56.1%, 59.7% and 74.7%, respectively. Figure 4b shows that the RBC occupancy further increased during the 1 h infusions of 0.25 and 0.5 mg h^{-1}, whereas the occupancy at the end of the 15 min infusion of 1, 1.5 and 2.5 mg was higher than that during the subsequent 1 h infusions. At the end of the 15 min infusion of 1, 1.5 and 2.5 mg, the RBC occupancy averaged 97.7%, 98.9% and 99.2%, respectively. The mean occupancy at the end of the 1 h infusion of 1 mg h^{-1}, 1.5 mg h^{-1} and 2.5 mg h^{-1} was 95.9%, 97.7% and 98.7%, respectively.

Figure 5 depicts the mean ABI as a function of the mean RBC occupancy of draflazine. It is clear that no 1:1 relationship was present between both endpoints. Figure 5 further shows that the relationship between the ABI and the RBC occupancy was similar for all infusion schemes of draflazine.

Discussion

The typical value for K_d shows that 50% of all nucleoside transporters are occupied by draflazine at a plasma concentration of 0.385 ng ml^{-1} (0.64 nmol l^{-1}). The proof for the capacity-limited high-affinity binding of draflazine to the human erythrocytes was previously established *in vitro* by Böhm *et al.* [20]. In this study with isolated human erythrocyte membranes, the specific red blood cell binding parameters of draflazine were determined in a displacement study with the nucleoside analog [^{3}H]-nitrobenzylthioinosine. The capacity-limited specific binding of draflazine to the nucleoside transporters located on the red blood cells was confirmed *in vivo* by Snoeck *et al.* [18]. We previously reported a more than two-fold higher *in vivo* K_d of 0.87 ng ml^{-1} plasma (1.45 nmol l^{-1}), which was still in the range of the individual Bayesian estimates of the present population analysis. This K_d was based on the pooled plasma and mean blood concentrations of eight subjects after a single 15 min i.v. infusion of 2.5 mg draflazine. The present typical value of K_d was very accurately estimated by NONMEM (CV 3.5%) and most likely approaches more closely the 'real' dissociation constant as the whole range of the $C_{RBC} - C_p$ relationship was covered in this study. In addition, more subjects and more infusion schemes were used for the population estimate. Finally, the interindividual variability in K_d of the healthy subjects was low (CV 13.1%) and was accurately estimated (CV 8.9%).

The typical value of the B_{max} of the specific binding of draflazine to the nucleoside transporters located on the erythrocytes was 158 ng ml^{-1} RBC, and was very precisely estimated by NONMEM (CV 2.1%). The population B_{max} was similar to the previously found value of 164 ng ml^{-1} RBC [18]. In addition, as 1l of human blood contains 5×10^{12} erythrocytes, it can be calculated from B_{max} and the molecular weight of draflazine (604.53) that each erythrocyte has about 14000 nucleoside transporters, which is in agreement with the previously reported *in vitro* binding data [19, 28–30]. The typical magnitude of the non-specific binding constant $K_{non\text{-}specific}$ was 0.615, demonstrating that the non-specific binding was about 0.3% of the total binding at K_d and about 23% at 80 ng ml^{-1} plasma. The non-specific binding constant was almost two-fold higher than that found in the previous study [18]. From equation 3 it is clear that, for a similar $C_{RBC} - C_p$ relationship, a lower population estimate of K_d will result in a higher typical value of the non-specific binding constant.

The intra-individual or unexplained variability was well described by the population parameters expressing the proportional and additive error of the model. These population parameters indicate that the intra-individual variability was low in this uniform group of healthy subjects (Table 2). However, the non-linear red blood cell partitioning of draflazine in the more diverse group of patients undergoing a CABG may be different and may be influenced by the disease status of the patient.

The specific binding to the erythrocytes appeared to be a source of non-linearity in the pharmacokinetics of draflazine. The total plasma clearance of draflazine slightly decreased with increasing doses, whereas the total clearance in whole blood increased with increasing doses (Table 3). The pharmacokinetics of draflazine in plasma and whole blood were not predictable from the pharmacokinetic data obtained in the previous study. Moreover, within this study, the disposition of draflazine following a certain infusion scheme could not be extrapolated from the data of the other infusion schemes. The impact of the specific erythrocyte binding on the pharmacokinetics of draflazine could be further explored by a model that integrates both pharmacokinetic and binding phenomena. An additional study with longer infusion durations of draflazine will allow to further develop this pharmacokinetic binding model and to investigate the steady-state pharmacokinetics of the drug.

For each individual subject, the relationship between the *ex vivo* measured ABI and the concentration of draflazine in plasma and whole blood could be characterized on the basis of a sigmoidal E_{max} model equation (Figure 3). The difference in IC_{50} and γ between the draflazine plasma- and blood- % ABI relationship might also be explained by the non-linear red blood cell partitioning. Typical values of E_{max}, IC_{50} and γ in whole blood were very precisely estimated by NONMEM (Table 4). In plasma, the precision of the IC_{50} was somewhat larger (9.3%), which could be explained by a larger interindividual variability (45.1%). With the exception of the IC_{50} in plasma, the parameters of the sigmoidal E_{max} equation in plasma and whole blood were similar to the previously reported values [18]. The typical value of the IC_{50} in plasma was about three-fold lower than the previously reported IC_{50} of 10.5 ng ml^{-1}. The present population estimate of IC_{50} of 3.76 ng ml^{-1} most likely approaches more closely the 'real' IC_{50} in plasma as the whole range of the ABI-C_p relationship was covered in this study.

The *ex vivo* measured ABI was a useful pharmacodynamic endpoint as the measured inhibition of the overall breakdown of adenosine possibly leads to a prolongation of the pharmacological effects of adenosine. The RBC occupancy did not coincide with the measured *ex vivo* ABI (Figure 5). However, the observed relationship was similar for all infusion schemes. Figure 5 depicts that the erythrocytes have to be occupied for 90% or more in order to obtain a substantial inhibition of the breakdown of adenosine. This can easily be explained by the huge preponderance of transport capacity *vs* enzymatic breakdown capacity. It is only at a relatively high RBC occupancy of draflazine that the nucleoside transport process rather than the enzymatic breakdown becomes the rate limiting step for the overall breakdown of adenosine. The overall catabolism of adenosine is the result of an initial transport of adenosine into the erythrocytes followed by deamination of adenosine by adenosine deaminase producing inosine, and finally a breakdown of inosine to hypoxanthine by purine nucleoside phosphorylase. The nucleoside transporter as well as the enzymes do have their own specific kinetic parameters making the kinetics of the overall breakdown of adenosine relatively complicated.

Previously, Van Belle *et al.* [14] demonstrated that a substantial inhibition of the breakdown of adenosine by draflazine largely prevented cardiac damage and death in catecholamine challenged rabbits. To obtain a substantial ABI in CABG patients, the RBC occupancy should be as high as possible. However, a complete occupancy should be avoided as this may lead to systemic accumulation of adenosine and possibly resulting in unwanted side-effects [18]. For these reasons, a 15 min i.v. infusion of 1 mg immediately followed by an infusion of 1 mg h^{-1} could be justified as the appropriate infusion scheme. An infusion rate of 1.5 or 2.5 mg h^{-1} could possibly result in a systemic accumulation of adenosine in some patients, making the favoured therapeutic window relatively small. Moreover, as the RBC occupancy rapidly declined after cessation of the 1 h infusion, it is expected that draflazine has to be infused during the whole period of risk in order to have the optimal anti-ischaemic protection. Studies with longer infusion durations will be needed to further investigate the RBC occupancy and the individual variability in the RBC occupancy during and after these long infusions, so that the dose rationale can be further founded.

We conclude that the specific binding of draflazine to the nucleoside transporters located on the erythrocytes was a source of non-linearity in the pharmacokinetics of draflazine. The estimate of the population parameter typical value of the specific binding constant K_d was 0.385 ng ml^{-1} plasma (0.64 nmol l^{-1}), demonstrating the high affinity of this binding. The percentage of red blood cell nucleoside transporters occupied by draflazine should be at least 90% in order to substantially inhibit adenosine breakdown *in vivo*. Finally, based on the percentage red blood cell occupancy of draflazine, it is suggested that a 15 min i.v. infusion of 1 mg draflazine immediately followed by an

infusion of 1 mg h^{-1} could be justified in patients undergoing a coronary artery bypass grafting in order to have an optimal anti-ischaemic protection.

Some data from this study have been presented at the Dutch Society of Clinical Pharmacology and Biopharmacy Meeting, March 1995 [31].

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