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Interspecies differences in plasma protein binding of MS-275, a novel histone deacetylase inhibitor

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Abstract MS-275 (MS-27–275; 3-pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl-carbamate) is a histone deacetylase inhibitor under clinical development as an anticancer agent. Here, we examined the role of protein binding as a possible determinant of the pharmacokinetic behavior of MS-275. The distribution of MS-275 in plasma was studied in vitro using equilibrium dialysis and ex vivo in five cancer patients receiving the drug orally at a dose of 10 mg/m^2. The dialysis method uses a tracer amount of [$G-^3$H]MS-275 on a 96-well microdialysis plate with a 5-kDa cut-off membrane, and requires 250 µl sample. The time to equilibrium was established to be within 5 h, and the mean unbound fraction of MS-275 (f_u) over a presumed therapeutic concentration range in healthy volunteer human plasma was 0.188 ± 0.0075 as compared to 0.168 ± 0.0144 in cancer patients. The binding was concentration-independent, indicating a low affinity, possibly non-specific and non-saturable process. MS-275 was found to bind in decreasing order to plasma > α_1-acid glycoprotein > albumin. Among 19 tested drugs, a slightly increased f_u was observed in the presence of only ibuprofen (f_u, 0.236 ± 0.001) and metoclopramide (f_u, 0.270 ± 0.042), suggesting weakly competitive displacement from protein-binding sites ($P < 0.01$). Compared to humans, f_u was significantly higher in plasma from mouse (0.376), rat (0.393), rabbit (0.355), dog (0.436), and pig (0.439) ($P < 0.01$), which may explain, in part, the species-dependent pharmacokinetic profile of MS-275 observed previously.

Keywords MS-275 · Histone deacetylase inhibitor · Protein binding · Equilibrium dialysis

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Introduction

Acetylation and deacetylation of histones plays an important role in the regulation of gene transcription and in the modulation of chromatin structure [7, 9]. In recent years, various agents have been identified that inhibit histone deacetylase activity and induce cell growth arrest, differentiation and/or apoptotic cell death [10]. MS-275 (MS-27–275; 3-pyridylmethyl-*N*-{4-[(2-aminophenyl)-carbamoyl]-benzyl-carbamate; Fig. 1) is a highly potent histone deacetylase inhibitor that also induces the expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ and gelsolin, and changes the cell cycle distribution [13, 15]. MS-275 has shown antiproliferative activity in various in vitro and in vivo human tumor models [8, 13], and is currently being tested in clinical trials involving patients with solid tumors or hematological malignancies. A preliminary pharmacokinetic evaluation of MS-275 given orally to cancer patients has shown that the terminal half-life of MS-275 in plasma (approximately 50 h) is substantially longer than that observed in laboratory animals (approximately 1 h) [12]. The basis for this long half-life in humans is possibly related to enterohepatic recirculation processes. However, a variety of other factors may influence the prolonged circulation of MS-275 in humans, including binding of the compound to plasma proteins. Indeed, drugs with high affinity for plasma proteins often demonstrate a relatively slow distribution and elimination of

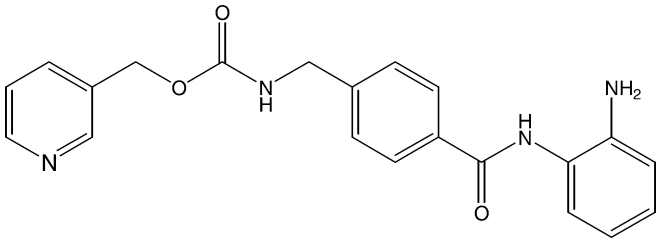

Fig. 1 Chemical structure of MS-275

drug from the central compartment, which may prolong the apparent half-life [14]. The purpose of this study was to characterize the binding properties of MS-275 to human plasma and individual proteins using a novel microequilibrium dialysis method, and to evaluate potential interspecies differences in binding affinity that might help explain the apparent pharmacokinetic discrepancy between humans and laboratory animals.

Materials and methods

Chemicals and reagents

MS-275 (batch number: 81300002; chromatographic purity, 99.82%) and [$G-^3$H]MS-275 (specific activity, 1543.6 MBq/mg) were kindly supplied by Schering AG (Berlin, Germany). HPLC-grade methanol and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ, USA). Ammonium formate and formic acid were purchased from Sigma (St. Louis, MO, USA). Deionized water was generated with a Hydro-Reverse Osmosis system (Durham, NC, USA) connected to a Milli-Q UV Plus purifying system (Marlborough, MA, USA). Bio-Safe II scintillation fluid was obtained from Research Products International (Mount Prospect, IL, USA). Purified human proteins, including albumin, α1-acid glycoprotein (AAG), α-, β-, and γ-globulin, fibrinogen, and lipoproteins, as well as human, mouse, rat, rabbit, dog, and pig plasma were obtained from Sigma. Other chemicals were of reagent grade or better. Pure protein solutions at respective physiological concentrations were prepared in 0.01 M phosphate buffer (pH 7.4). The stock solutions of all test substances were made in dimethylsulfoxide. Human blood was also obtained from healthy volunteers or cancer patients receiving MS-275, and the plasma fraction was separated by centrifugation (3,000*g* for 5 min at 37°C), and used within 1 h after collection. Frozen, drug-free heparinized human plasma was obtained from the National Institutes of Health Clinical Center Blood Bank (Bethesda, MD, USA).

Development and validation of dialysis method

Equilibrium dialysis was performed on a plate rotator (Model # 74–2334, Harvard Apparatus, Holliston, MA, USA) at 37°C in a humidified atmosphere of 5% CO_2 using 96-wells micro-dialysis plates (Harvard Apparatus) [1]. The dialysis compartments in each well are separated by a regenerated cellulose membrane with a 5-kDa cut-off. Experiments were carried out with 250 µl aliquots of plasma containing a tracer amount of [$G-^3$H]MS-275 against an equal volume of 0.01 M phosphate buffer (pH 7.4). Drug concentrations in 125 µl-aliquots of both compartments were measured by liquid scintillation counting for 1 min following the addition of Bio-Safe II scintillation fluid on a Model LS6000IC counter (Beckman Instruments, Inc., Columbia, MD).

To evaluate the specificity of this procedure and check for preliminary evidence of displacement effects of other drugs on protein binding of MS-275, blank human plasma was spiked with 19 different commonly administered drugs at a fixed concentration of 1 µg/ml and was analyzed for changes in the fraction unbound drug (f_u). The accuracy and precision were assessed by analyzing quadruplicate samples prepared from five different plasma sources in quintuplicate on five separate occasions. Within- and between-assay precision estimates were obtained by one-way analysis of variance, and reported as relative standard deviation. The impact of stability of MS-275 protein binding in human plasma was assessed during a freeze-thaw cycle at room temperature after 24 h.

In vitro binding experiments

Preliminary experiments indicated that volume shifts during the dialysis period were negligible ($< 10\%$), and hence the results were used directly without applying a correction factor. Furthermore, a direct comparison of human citrated lyophilized plasma obtained from Sigma with freshly obtained human plasma indicated, under identical experimental conditions, no significant difference in binding of MS-275 ($P = 0.21$). The time course of equilibrium was assessed in quadruplicate at 15 and 30 min, and at 1, 2, 3, 4, 5, 6, 22, 24 and 28 h after start of the experiment. Since f_u measurements were to be made on patient samples that contained variable amounts of drug, f_u was also determined in plasma samples over the anticipated clinically relevant concentration range of MS-275 (i.e., 0, 1, 5, 10, 50, 100 and 500 ng/ml).

Estimation of binding parameters

The drug concentration ratio in the buffer and plasma or protein solution after dialysis was calculated for each paired observation, and was taken as an estimate of the unbound drug fraction (f_u). The bound drug fraction (f_{bd}) was calculated as $f_{bd} = (1 - f_u) \times 100\%$.

Modified Scatchard plots were constructed using the bound drug concentration (C_{bd}) and the unbound drug concentration (C_u), and initial estimates of binding parameters were obtained using an automated-model selection procedure implemented in the Siphar v4.0

software package (InnaPhase, Philadelphia, PA, USA). For human albumin and AAG, the observed data were described by equations for saturable and non-saturable binding [$C_{bd} = (nK) \times C_u$]. In these equations, C_{bd} and C_u are expressed as molar concentrations, and nK the contribution constant of nonspecific, non-saturable binding on one site (per molar concentration of protein). Binding parameters were calculated by an iterative nonlinear regression analysis using the Powell minimization algorithm and weighted least squares with a weight equal to $1/y$. The models were evaluated by the Akaike Information Criterion, weighted sum of squared deviations and the coefficient of variation for each parameter estimate.

Patients and treatment

Blood samples were available from five patients, who were enrolled onto a Phase I clinical study with MS-275 as single-agent therapy [12]. Individual drug doses were normalized to body-surface area, and were administered orally as capsules (Schering AG) with food at a dose of 10 mg/m^2. Trial design, inclusion and exclusion criteria, premedication regimens, and detailed clinical profiles are documented elsewhere. The clinical protocol was approved by the National Cancer Institute review board (Bethesda, MD, USA), and all patients provided written informed consent before entering the study. From each patient, serial plasma samples were obtained during the first course of treatment at the following time points: (1) immediately before drug administration (pre-dose), and (2) at 0.5, 1, 2, 6, 12, 24, 48, 60, 72, and 84 h after the first drug administration. All blood samples were immediately placed in an ice-water bath, centrifuged within 30 min of collection at 1,000*g* for 10 min at 4°C, and were stored at or below −70°C until analysis (see below).

Measurement of total drug concentrations

Total MS-275 concentrations were determined using a validated analytical method based on liquid chromatography coupled with mass spectrometric detection [6]. Chromatography was carried out with a HP1100 system (Agilent Technology, Palo Alto, CA, USA). Data were acquired and integrated by the ChemStation software run on a HP Vectra 150/PC with a Windows NT operating system. Calibration curves ranged from 1 to 100 ng/ml, and were analyzed using a weight factor proportional to the nominal concentration. Sample pretreatment involved a one-step protein precipitation with acetonitrile of 0.1-ml samples. The analysis was performed on a stainless steel column (75×4.6 mm I.D.) packed with 3.5-μm Phenyl-SB material (Agilent Technology), using methanol-10 mM ammonium formate (pH = 3) (55:45, vol/vol) as the mobile phase. The lowest limit of quantitation was determined to be 1 ng/ml and the values for precision and accuracy were always ≤ 5.58% and < 11.4% relative error, respectively. The method was successfully applied to examine the pharmacokinetics of MS-275 in a cancer patients.

Measurement of unbound drug concentrations

The fraction unbound (f_u) MS-275 in each individual patient plasma sample was determined using equilibrium dialysis, and samples were analyzed for total radioactivity (i.e., [$G-^3$H]MS-275) by liquid-scintillation counting as described above. The unbound drug concentrations (C_u) were calculated from the fraction unbound drug (f_u) and the total drug concentration in plasma (C_p) (i.e., the total of unbound and protein bound), as $C_u = f_u \times C_p$.

Pharmacokinetic analysis

Estimates of pharmacokinetic parameters for total and unbound MS-275 in plasma were derived from individual concentration-time data sets by noncompartmental analysis using the software package WinNonLin v4.0 (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method from time zero to the time of the final quantifiable concentration (AUC[tf]). The AUC was extrapolated to infinity by dividing the last measured concentration by the rate constant of the terminal phase (k), determined by log-linear regression analysis. The apparent oral clearance of MS-275 (CL/F) was calculated by dividing the administered dose by the observed AUC[inf], and the terminal half-life was calculated as $ln2 / k$.

Statistical considerations

All experiments were performed in triplicate on at least three separate occasions, and statistical analyses were carried out using NCSS v2001 (J.L. Hintze, Kaysville, UT, USA). The effects of MS-275 concentration, concomitant drugs, and protein source on drug binding were estimated by a one-way ANOVA, and if overall $P < 0.05$, then followed by the Tukey-Kramer post-hoc test. All data are presented as mean values ± standard deviation (SD), unless stated otherwise, and for all tests the a priori cutoff for statistical significance was taken at P-value < 0.05.

Results

Validation of dialysis method for MS-275

Preliminary experiments revealed that the time to equilibrium was attained around 5 h (Fig 2). It was confirmed in all equilibrium dialysis experiments that the total drug recovery from the fractions was equal to the

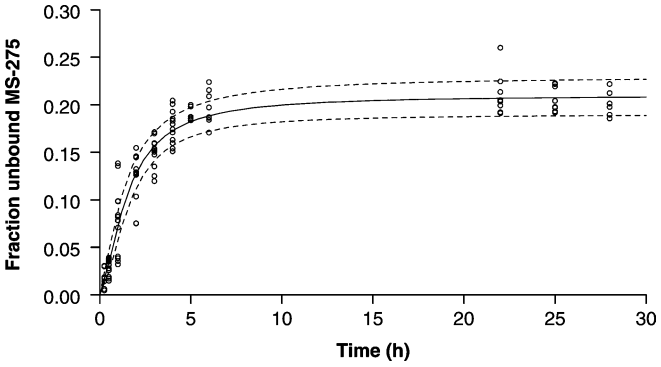

Fig. 2 Time course to reach equilibrium for the fraction unbound MS-275. Data are presented as individual observations (*symbols*) and a predicted model fit according to a modified Hill function ($R^2 = 0.930$) (*solid lines*) with 95% prediction intervals (*dotted lines*)

amount of [$G-^3$H] MS-275 added to the plasma samples (mean recovery, 98%; $P > 0.05$ versus hypothesized mean of initial value = 100%). The mean relative SD of all sample values was less than 10%, assuring high discriminatory power in the detection of changes in MS-275 f_u in patient samples. With the final method, the within-run and between-run variability were always less than 6.4% and 9.8%, respectively.

Fresh plasma from five different sources was analyzed for f_u in triplicate and then the same samples were frozen and thawed at room temperature on the next day and immediately analyzed to determine f_u. The mean f_u values were 0.188 and 0.206, respectively, before and after the freeze-thaw cycle ($P > 0.05$), suggesting no significant influence. In separate experiments using the LC-MS assay, the chemical stability of MS-275 during the dialysis was confirmed by analysis of plasma samples spiked with 100 ng/ml of MS-275 in dialysis plates after incubation for 5 h at 37°C (data not shown).

In vitro binding interactions with MS-275

MS-275 was found to bind moderately to human plasma (mean, 81.2 ± 3.2%), with a free drug fraction of 0.188 ± 0.008. There was no significant source difference in f_u when plasma was used from different healthy individuals (mean f_u, 0.185; $P = 0.0938$). The f_u obtained in previously frozen plasma from healthy volunteers was found to be slightly higher than that observed in the plasma from six cancer patient (mean f_u, 0.188 versus 0.168; $P = 0.113$).

At clinically relevant concentrations of MS-275 (1–500 ng/ml), the binding was concentration-independent ($P > 0.05$), indicating a low-affinity, possibly non-specific and non-saturable process. MS-275 binding to physiological levels of albumin (3.5–4.5 g/dl; f_u, 0.27 ± 0.042) and AAG (0.04–0.1 g/dl; f_u, 0.19 ± 0.0037) was similar, drug-concentration independent ($P = 0.53$ and $P = 0.80$, respectively), and similar to the binding to patient plasma (Fig. 3). When albumin and AAG were combined in the same buffer, the mean f_u was 0.146 ± 0.0010, suggesting that albumin and AAG contribute to the majority of binding of MS-275 in human plasma. Regression modeling revealed that the weak binding to albumin and AAG was non-saturable on a single site in the concentration range studied, with the bound concentration linearly related to unbound drug ($R^2 > 0.99$). Binding affinity to AAG was about 4.7-fold higher than that of albumin, with association constants for non-saturable binding (nK) of 0.0247 ± 0.0003 μM^{-1} and 0.116 ± 0.020 μM^{-1} for albumin and AAG, respectively. Subsequent experiments indicated that MS-275 had weak binding affinity for globulins (α, β, γ), fibrinogen, and high and low-density lipoproteins (Fig. 3).

Displacement interactions on binding sites

A slightly increased f_u was observed in the presence of ibuprofen (f_u, 0.236 ± 0.001) and metoclopramide (f_u, 0.270 ± 0.042), suggesting weakly competitive displacement from protein-binding sites ($P = 0.00012$, one-way ANOVA) (Table 1). The other tested drugs did not significantly alter the protein binding of MS-275. It should be noted, however, that these concentrations could be less than clinically relevant for select agents.

Interspecies differences in MS-275 binding

MS-275 demonstrated a striking interspecies difference in plasma protein binding ($P = 0.00846$, one-way ANOVA) (Fig. 4); compared to human plasma, the binding of MS-275 was significantly reduced in the mouse (f_u, 0.378 ± 0.101), rat (f_u, 0.393 ± 0.0070), rabbit (f_u, 0.375 ± 0.0416), dog (f_u, 0.436 ± 0.0159), and pig plasma (f_u, 0.439 ± 0.0116).

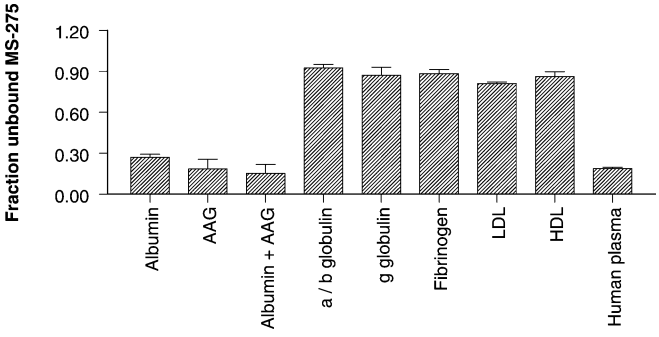

Fig. 3 Binding of MS-275 to human plasma proteins. Data are presented as mean values (*bars*) ± SD (*error bars*). *AAG* α_1-acid glycoprotein; *LDL* low-density lipoprotein; *HDL* high-density lipoprotein

Table 1 Effect of potentially co-administered drugs on plasma binding of MS-275

Drug	Fraction unbound MS-275		
	Mean	SD	Percent change versus control (*P*-value)
Acetylsalicylic acid	0.187	0.0163	+6.58 ± 5.35 (NS)
Alendronate	0.159	0.0124	−9.33 ± 4.06 (NS)
5-Azacytidine	0.178	0.0163	+1.40 ± 5.36 (NS)
Caffeine	0.221	0.0185	+25.8 ± 6.07 (NS)
Celecoxib	0.162	0.0447	−22.4 ± 14.7 (NS)
Cyclosporin A	0.178	0.0087	−0.389 ± 2.87 (NS)
Dexamethasone	0.182	0.0327	+14.1 ± 10.7 (NS)
Docetaxel	0.168	0.0099	−4.16 ± 3.26 (NS)
Erythromycin	0.164	0.0113	−6.48 ± 3.72 (NS)
Fludarabine	0.163	0.0095	−7.10 ± 3.13 (NS)
Hydrocortisone	0.170	0.0038	−3.18 ± 1.24 (NS)
Ibuprofen	0.236	0.0013	+34.6 ± 0.443 (0.00162)
Ketoconazole	0.173	0.0075	−1.83 ± 2.47 (NS)
Metoclopramide	0.247	0.0243	+40.8 ± 8.00 (0.00327)
Midazolam	0.174	0.0082	−0.808 ± 2.70 (NS)
Nifedipine	0.178	0.0139	+1.19 ± 4.57 (NS)
Paclitaxel	0.168	0.0083	−7.11 ± 2.74 (NS)
Ritonavir	0.177	0.0010	+0.817 ± 0.00 (NS)
UCN-01	0.169	0.0035	−3.94 ± 1.14 (NS)

NS not significant

Clinical pharmacokinetics of unbound MS-275

The developed equilibrium dialysis method was next applied to prospectively define the concentration-time profiles of total and unbound MS-275 in five patients with cancer receiving single-agent MS-275, which was administered orally at a dose of 10 mg/m^2. The mean plasma concentration-time profiles for total and unbound MS-275 are shown in Fig. 5a. A summary of the pharmacokinetic parameters for total and unbound MS-275 is provided in Table 2. Moderate inter-individual variability in unbound MS-275 pharmacokinetic parameters was noted at the 10 mg/m^2 dose level, with a coefficient of variation for the apparent oral clearance of 36%. In vivo, there were no significant changes in extent of MS-275 binding with 79.7% (f_u, 20.3 ± 5.88) ($n = 56$) drug bound based on data obtained at individual sampling time-points (Fig. 5b).

Discussion

In the present study we have described the in vitro and ex vivo plasma protein binding of MS-275, an investigational histone-deacetylase inhibitor. The binding of MS-275 to human plasma was approximately 81% and independent of drug concentration over the full presumed clinically relevant range. When binding studies were extended to individual proteins, it was found that AAG and human serum albumin contributed to about an equal extent to drug binding, with an association constant for nonspecific, nonsaturable binding of 0.116 μM^{-1} and 0.0247 μM^{-1} for AAG and albumin, respectively. There was a slight increase in value of f_u obtained from healthy volunteer plasma compared to plasma from six cancer patients. Even though this difference was not statistically significant, the decrease in overall f_u may be due to the plasma protein level changes that occur in cancer patients, including decreased albumin [11] and increased AAG [2].

Since treatment with MS-275 commonly involves numerous other drugs, displacement of bound MS-275 by these agents might occur, particularly in view of the relatively weak associations with its main binding proteins, AAG and albumin. The effect of 19 potentially co-administered drugs with MS-275 on its binding to plasma was performed at a fixed concentration of 1 μg/ml. It was found that only ibuprofen and metoclopramide at relevant clinical concentrations, significantly increased f_u of MS-275. Previous investigations have shown that albumin is the major binding protein in plasma for ibuprofen [17]. Furthermore, statistically significant interactions involving displacement of drugs from binding sites on albumin by ibuprofen have been described for various drugs [4]. However, based on theoretical considerations outlined in detail elsewhere [3], it is unlikely that changes in the protein binding of MS-275 as a result of co-administration of ibuprofen in vivo will significantly influence the systemic exposure to MS-275. Hence, in spite the preliminary data seem to indicate that MS-275 might have a rather narrow therapeutic concentration range [12], careful monitoring of combined use of MS-275 and ibuprofen is not indicated. The increase in f_u in the presence of metoclopramide is less well understood. The main binding protein for metoclopramide in human plasma is AAG [16], but its affinity is negligible compared with UCN-01 [5], which agent does not substantially interfere with the binding properties of MS-275. Given the structural similarities of metoclopramide and MS-275, it is possible that the observed interaction involves competition for the same site on an as yet unidentified plasma protein. The other tested agents had no substantial influence on the binding of MS-275, even at relatively high concentrations, and are thus unlikely to modulate the pharmacokinetic profile of MS-275 in vivo.

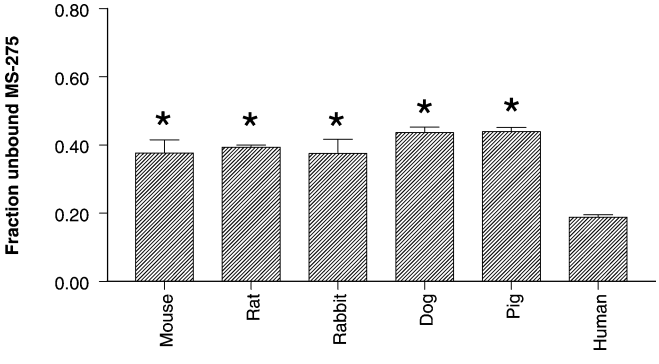

Fig. 4 Interspecies comparison of MS-275 binding to plasma. Data are presented as mean values (*bars*) ± SD (*error bars*), and the star (asterisks) indicates $P < 0.05$ versus human plasma

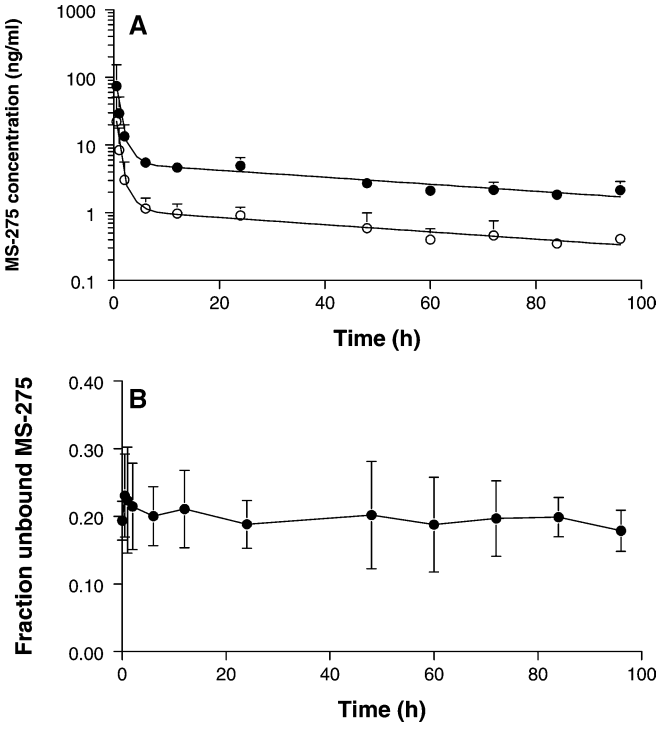

Fig. 5 Concentration-time profiles of total MS-275 (ng/ml) (*closed circle*) and unbound MS-275 (ng/ml) (*open circle*) in plasma (**a**) and the fraction unbound MS-275 in plasma versus time profiles (**b**). Data are presented as mean values (*symbols*) ± SD (*error bars*), and were obtained from five patients with cancer treated with MS-275 given orally at a dose of 10 mg/m^2

The present study demonstrated a striking species-dependence of plasma protein binding of MS-275. There was a remarkable two-fold difference in plasma protein binding of MS-275 to human plasma as compared to plasma from a variety of other species that are commonly used for pre-clinical studies. The reasons for the large differences in binding of MS-275 between the tested species are currently unknown, although one possibility is species-dependent binding of MS-275 to AAG, as has been described previously for various xenobiotic ligands [18], including the staurosporine analogue UCN-01 [5]. Regardless of the exact underlying mechanism, this species dependent binding of MS-275 should be taken in consideration when attempting to extrapolate data obtained in tumor-bearing animals to the clinical situation. Because unbound drug is involved in distribution and systemic elimination [14], the differential binding of MS-275 might explain, at least in part, the relatively slow apparent oral clearance and the long terminal half-life of observed in humans (~50 h) [12], in comparison with the laboratory animals (mice 1.1 h, rats 2.1 h, dogs < 1 h) (Schering AG, data on file). However, additional mechanisms cannot be excluded.

Table 2 Summary of pharmacokinetic parameters[a]

Parameter	Total MS-275	Unbound MS-275
C_{max} (ng/ml)	50.6 ± 64.6 (9.41–163)	7.25 ± 7.18 (1.75–19.1)
T_{max} (h)	1.0 (0.50–2.0)	1.0 (0.50–2.0)
AUC (ng·h/ml)	476 ± 155 (360–747)	96.6 ± 41.7 (56.2–167)
CL/F (l/h/m^2)	22.4 ± 5.43 (13.4–27.8)	117 ± 42.1 (59.8–178)
$T_{1/2}$ (h)	46.4 ± 12.6 (27.7–60.3)	52.4 ± 18.8 (30.3–80.5)
AUC ratio C_u/C_p	N/A	0.20 ± 0.042 (0.14–0.25)

[a]Data were obtained from five patients receiving MS-275 orally at a dose of 10 mg/m^2, and are presented in the table as mean values ± SD with range in parentheses, except for T_{max} (median)C_{max} peak plasma concentration; T_{max} time to peak concentration; AUC area under the plasma concentration-time curve; CL/F apparent oral clearance; $T_{1/2}$ half-life of the terminal phase; C_u unbound drug concentration; C_p total drug concentration in plasma

There was moderate inter-individual variability in unbound MS-275 pharmacokinetic parameters at the dose level tested, with a relatively lower coefficient of variation for the apparent oral clearance, which suggests that the inter-individual variation in plasma protein binding of MS-275 is relatively small in metabolically normal individuals. Consistent with the in vitro data, almost 80% of drug was bound within the circulation without any trend over time. Therefore, protein binding does not seem to be an important consideration in pharmacokinetic monitoring for MS-275 in cancer patients, and that the more easily measured total MS-275 concentrations provide a consistent and accurate reflection of the unbound concentrations with little interpatient variability (i.e., the binding is concentration independent and reversible).

In conclusion, a reliable and reproducible equilibrium dialysis method for the determination of the fraction unbound MS-275 in plasma was developed and validated. MS-275 was found to bind with a moderate degree of affinity to several human plasma proteins, including AAG and albumin. This clearly signifies the importance to account for differences in the fraction unbound drug when attempting to extrapolate data obtained in in vitro model systems in protein-free media to the clinical situation. The plasma binding of MS-275 was also found to be significantly species dependent. Indeed, whereas in humans the major fraction of the administered drug is sequestered by AAG and albumin, thereby restricting the unbound concentration and affecting distribution and elimination pathways, in the other tested species binding of MS-275 to plasma proteins was relatively insignificant. This not only provides a mechanistic explanation for species differences in pharmacokinetic parameters of MS-275 noted previously, but also suggests that interspecies relationships between drug exposure measures and pharmacodynamic outcome of treatment should be based on unbound MS-275 concentrations.

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