Abstract

N-(6-Chlorophenoxyhexyl)-N′-cyano-N′′-4-pyridylguanidine (CHS 828) is a novel anticancer agent that shows schedule-dependent effects in vitro and in vivo, as well as in Phase I clinical trials. A rat hollow fibre model was used to investigate whether this dependency is due to pharmacokinetic and/or pharmacodynamic factors. The effect on two cell lines, MDA-MB-231 (breast cancer) and CCRF-CEM (leukaemia) were studied after CHS 828 was administered orally as a single dose or in a 5-day schedule, at different total dose levels. The 5-day schedules were associated with greater effects on both cell lines compared with single doses. A one-compartment pharmacokinetic model, with a half-life of 2.3 h and a consecutive zero- and first-order process to describe dissolution and absorption, fit the concentration data. Pharmacokinetics were dose-dependent, as the fraction absorbed decreased with increasing dose. Clearance increased with accumulative exposure. Twenty hours after administration, concentrations started to increase again, probably due to coprophagy. Pharmacokinetic–pharmacodynamic models characterized the cell growth and cell kill over time and showed that schedule-dependent antitumour effects were present also when the dose-dependent pharmacokinetics were accounted for. The prolonged schedules of CHS 828 were therefore associated with greater antitumour effects than single doses of the same total exposure.

1. Introduction

The choice of administration schedule for a drug will often affect its efficacy; changing the schedule of administration may have a greater effect on the treatment outcome than increasing the dose. This is particularly true for anticancer drugs, which typically have a narrow therapeutic window. For example, dependence on schedule has been shown for etoposide in the antitumour effects (Slevin et al., 1989) and for paclitaxel in the myelosuppressive effects (Eisenhauer et al., 1994). However, for 5-fluorouracil, part of the lower haematological toxicity associated with infusions, compared with bolus injections, can be explained by the non-linear pharmacokinetics of the drug, i.e. a bolus injection will produce a larger area under the concentration–time curve (AUC) than an infusion of the same total dose. When different administration rates produce the same total AUC, but cause different magnitudes of effect, the drug is schedule-dependent in the pharmacodynamics. To properly evaluate and describe a seemingly schedule-dependent pharmacodynamic effect, i.e. deviation from AUC-dependent effects, potential dose-dependence in the pharmacokinetics needs to be characterized.

N-(6-Chlorophenoxyhexyl)-N′-cyano-N′′-4-pyridylguanidine (CHS 828) is a novel anticancer agent with pronounced...
activity against tumour cells (Aleskog et al., 2001; Hassan et al., 2001a). However, the antitumour effects of CHS 828 have shown dependence on the administration schedule in both in vitro and in vivo studies. The in vitro potency of CHS 828 increased with time of exposure (Hassan et al., 2001b). Weekly administrations of CHS 828 were superior to daily administrations in a xenograft model in mice (Vig et al., 2001b). However, the antitumour effects of CHS 828 increased with time of exposure (Hassan et al., 2001). In addition, schedule-dependent toxicity was present in phase I studies aiming to establish a maximum tolerated dose, as a higher total dose was tolerated for a single-dose regimen than for a 5-day dosage regimen (Horstadius et al., 2002; Ravaud et al., 2001).

Since CHS 828 is poorly soluble (0.1 mg/ml in a solution of 10% DMSO) and, at effective dosage levels, can only be administered orally either as a suspension or in capsules, the drug amount actually reaching the circulation may be variable. In addition, the pharmacokinetics at higher dose levels have not been investigated sufficiently to exclude dose-dependency. A pharmacokinetic-pharmacodynamic model is therefore desirable to separate the pharmacokinetic and pharmacodynamic factors contributing to the schedule-dependency. Although several pharmacokinetic-pharmacodynamic models that can estimate the time course of chemotherapy-induced myelosuppression have been published (Friberg and Karlsson, 2003), there are few reports on modelling the time course of chemotherapy-induced tumour effects (Jusko, 1971, 1973; Lobo and Balthasar, 2002; Simeoni et al., 2004). Only two of the examples describe the time course of tumour effects in vivo (Jusko, 1973; Simeoni et al., 2004). The latter example also illustrated the potential of pharmacokinetic-pharmacodynamic modelling of animal data in the development of anti-cancer drugs. However, for drugs with relatively high inter-individual variability (IVV) in pharmacokinetics, it is preferable that pharmacokinetics and pharmacodynamics are studied in the same animal and by using a population analysis approach IVV can be separated from residual variability.

Tumour regressions in animal experimental tumour models are considered an important endpoint of clinical relevance (Plowman et al., 1997). The hollow fibre model is a simple in vivo tumour model, where tumour cells are cultured in semi-permeable fibres, which are easily implanted and retrieved. It has mainly been used at the National Cancer Institute in nude mice as a screening model to reduce the number of time- and animal-consuming xenograft studies (Hollingshead et al., 1995). However, the hollow fibre model in immunocompetent rats (Jonsson et al., 2000) offers the potential for studying pharmacokinetic-pharmacodynamic relationships of anticancer drug effects over time, within the same animal. In addition, as several fibres/tumours can be evaluated in the same animal, IVV and residual variability can be separated when data are analyzed in non-linear mixed effects model programs, such as NONMEM.

The main aim of the present study was to reveal whether the previously observed schedule dependence of CHS 828 in vivo (Jonsson et al., 2000) is due to dose-dependent pharmacokinetics, due to tumour cell re-growth before effect evaluation for the single dose and/or due to a “true” schedule-dependent pharmacodynamic effect. This required more extended experimental studies with different dose levels and schedules of CHS 828, as well as studies of the antitumour effect over time. The rat hollow fibre model was chosen to this end. To be able to separate pharmacokinetic and pharmacodynamic factors contributing to the schedule dependence, this work also presents the development of a pharmacokinetic-pharmacodynamic model of the tumour effects.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats were obtained from Charles River, Uppsala, Sweden. They were acclimatized for at least 1 week before randomization and the start of the experiments. The rats had free access to food and water throughout the study. Phenobarbital was used for euthanasia. The Animal Ethics Committee in Uppsala approved the study (No. C67/99).

2.2. Drugs

CHS 828 was provided by LEO Pharma (Ballarup, Denmark). The drug was prepared in dark glass bottles as 5–50 mg/ml suspensions with methyl cellulose (1–11%) and Millipore water. Before administration, the suspensions were subjected to ultrasonic radiation for 30–60 min and mixed thoroughly with a magnetic stirrer. Control rats received a mixture of methyl cellulose and water.

2.3. The hollow fibre model and study design

The human breast cancer cell line MDA-MB-231 (Cailleau et al., 1974) and the human leukaemia cell line CCRF-CEM (Foley et al., 1965) were kind gifts from Dr. J. Bergh at the Department of Oncology, Uppsala University, Sweden, and Dr. W.T. Beck at the Department of Pharmacology, University of Tennessee, TN, respectively. The cells were maintained in RPMI-1640 culture medium supplemented with 10% calf serum, glutamine and streptomycin/penicillin (Sigma Chemical, St. Louis, MO), passaged twice a week and harvested with trypsin/EDTA (Biochrome, Berlin, Germany).

The hollow fibre procedure in immunocompetent rats has been described earlier (Jonsson et al., 2000) and is based on a method developed at the National Cancer Institute (Hollingshead et al., 1995). Briefly, cell suspensions were flushed into polyvinylidene fluoride (PVDF) hollow fibres
(500 kDa molecular weight cut-off, 1 mm inner diameter; Spectrum Medical Industries, Los Angeles, CA), the fibres were heat-sealed and cut at 20 mm intervals, and incubated in vitro at 37 °C in supplemented medium for 2 days prior to implantation. Eight fibres per rat were inserted subcutaneously under anaesthesia with 2.5% enflurane (Efrane; Abbott, Stockholm, Sweden) mixed with nitrous oxide and oxygen (1.5:1min).

The study was divided into seven experiments, with six rats in each; two rats received single doses of the drug, two received 5-day dosing regimens and two animals served as controls. Each rat carried four fibres of two different cell lines or eight fibres of the same cell line. Drug administration started in the morning the day after fibre implantation (Fig. 1). CHS 828 was administered by oral gavage as a single dose of 125, 250, 375 or 500 mg/kg or as a fractionated schedule with five consecutive daily administrations of equal amounts to make up the same total doses as those used as single doses. Corresponding vehicle solutions and schedules were given to the control rats. At the dose levels of 125, 250 and 500 mg/kg, two rats of each schedule were run in two different experiments, carrying a total of eight fibres per cell line. For the MDA-MB-231 series (MDII series; Beckman Coulter, Luton, UK) within 2 h after the first dose (Fig. 1). All blood samples were drawn from a hind paw vein after the rat had been placed on a heating pad for at least 10 min. Leukocyte counts, platelet counts and haemoglobin values were determined in a Coulter Counter (MDII series; Beckman Coulter, Luton, UK) within 2 h after sampling. As the 375 mg/kg dose did not show haematological toxicity (see Section 3), the blood counts were only followed in the first four (of eight) investigated rats of each schedule at this dose level. However, the haematological toxicity of the highest dose level (500 mg/kg) was investigated further in eight additional rats. Half of them received a single dose and half received a five-dose regimen.

At four individually pre-determined time points (range 10 min to 48 h after dosing), 250–300 μl blood was collected into eppendorf tubes. After 30–60 min at 4 °C, the blood was centrifuged at 7000 rpm for 10 min. The serum was immediately frozen and stored at −70 °C until analysis of CHS 828 concentrations.

The concentration of CHS 828 in serum was determined by reversed-phase HPLC with UV-detection as described earlier (Jonsson et al., 2000), in which all known metabolites are separated from CHS 828. Briefly, 50 μl rat serum was mixed with 950 μl blank serum prior to work up and EO 859-000 (LEO Pharma) was added to each sample as internal standard. Quantification was based on a CHS 828 spiked standard curve.
in the range of 50–20,000 ng/ml. The within- and between-day variability ranged from 8 to 15% and the accuracy was 91–97%.

2.6. Pharmacokinetic–pharmacodynamic modelling

Population pharmacokinetic and pharmacodynamic modelling was performed by fitting all data simultaneously, using the first order conditional estimation (FOCE) method with INTERACTION in the non-linear mixed effects model program NONMEM [versions V and VI beta; (Beal and Sheiner, 1992)]. The estimated population model parameters were fixed effects related to the typical individual, random effects, with magnitudes of inter-individual variability (IIV) in parameters and residual variability (proportional and/or additive) between individual predictions and observations. The difference in the objective function values (OFV) produced by NONMEM was used to discriminate between nested models, as this difference is approximately $\chi^2$, distributed. A significance level of $p < 0.05$ was applied for a one-parameter difference, which corresponds to a drop in OFV of 3.84. The appropriateness of a model to describe the data was based on goodness of fit, the parameter estimates and their corresponding standard errors. The S-PLUS (Version 2000, Insightful, Seattle, WA) based program Xpose 3.0 (Jonsson and Karlsson, 1999) was used for model diagnostics.

In addition to the rats from the fibre studies, another 20 rats were included in the pharmacokinetic analysis, for which four to six blood samples per rat were drawn after administration of total doses in the range of 20–500 mg/kg (5.9–156 mg), either as a single dose or in a five-dose regimen. The pharmacokinetic sampling and drug assay methodology were as above. In eight of these rats, samples were also taken on the fifth day of administration, to examine potential changes in drug disposition over time.

Log-transformed concentrations were used in the model building and compartment models with linear and non-linear elimination were tried, as well as various absorption models with and without dose-dependencies. Individual concentration–time profiles based on empirical Bayes estimates of pharmacokinetic parameters were used in the pharmacokinetic–pharmacodynamic modelling.

All cell density–time profiles, from all rats, were modelled simultaneously for each cell line, including the cell density data from control animals. Each rat in a specific experiment was modelled to have the same baseline value, i.e. all three to five OD measurements on the day of implantation were added for each rat in the data set. Consequently, inter-experimental variability in baseline measurements (ODBaseline) was estimated instead of inter-individual variability. Log-transformed OD values were used.

Of all the models considered, only the key models that gave reasonable predictions based on knowledge from previous in vitro studies are described here. Compared with many other anticancer drugs, CHS 828 shows a more delayed effect on cell viability. DNA and protein syntheses are unaffected during the first 24 h of CHS 828 exposure but are then abruptly inhibited (Martinsson et al., 2001). The cell kill rate is fairly constant and at 72 h from first exposure the cell survival index was 5% of control in a human lymphoma cell line (Martinsson et al., 2001). The schedule dependence observed in vitro has been modelled as a sharp decrease in EC50 that occurs after 24–29 h of exposure (Hassan et al., 2001b). The concentration–effect relationship associated with the high potency is very steep (Hassan et al., 2001b), indicating more or less an all or nothing phenomenon.

According to this, a threshold cumulative effect on the cells (AUEThresh) before cell-kill (Kill) starts was assumed in the modelling, i.e. the cell viability was unaffected until AUEThresh was attained. Exponential cell growth was applied and the differential equations of the change in OD over time were written as

$$\frac{d\text{OD}}{dt} = k_{\text{Grow}} \text{OD} \quad \text{for AUE}_{0-\gamma} < \text{AUE}_{\text{Thresh}}$$  (2)

$$\frac{d\text{OD}}{dt} = k_{\text{Grow}} (1 - \text{Kill}) \text{OD} \quad \text{for AUE}_{0-\gamma} \geq \text{AUE}_{\text{Thresh}}$$

AUE0−γ is the cumulative effect that CHS exerts on the cells from the time of first administration to time t.

$$\text{AUE}_{0-\gamma} = \int E(C)\,dt$$  (4)

E(C) describes the relationship between the serum concentration of CHS 828 and the effect on the cells. This function was tried in the model building to be a sigmoid $E_{\text{max}}$-model

$$E(C) = E_{\text{max}} \frac{C/y - EC_{50}}{C/y + EC_{50}}$$  (5)

or simplifications thereof; a linear model ($y = 1$ and $C_C = EC_{50}$), a basic $E_{\text{max}}$-model ($y = 1$) or a threshold-concentration model ($y = 100$). A four-parameter model for asymmetric concentration–effect curves, i.e. Richards model, was also tried, which can be simplified to the three-parameter Gompertz model (Richards, 1959; Van der Graaf and Schoenmaker, 1999). For a linear model, the effect is proportional to the concentration and independent of the schedule of administration. AUE0−γ can then be substituted by AUE0−γ (and AUEThresh by AUCThresh). Any deviation from the same linear concentration–effect relationship for both schedules indicates schedule dependence.

3. Results

3.1. Fibre cell net growth

For each dosing regimen and cell line, 6–8 fibres were available for effect evaluation 5 days after the first dose, except for the total doses of 375 mg/kg for MDA-MB-231
Fig. 2. Net growth for different dose levels after fibre retrieval, 5 days after first administration (left) and net growth over time for the 375 mg/kg doses (right) for MDA-MB-231 (A and B) and CCRF-CEM (C and D). In panels A and C each data point shows the mean ± S.E.M. from 6 to 8 fibres (16–17 fibres at the dose level of 375 mg/kg for MDA-MB-231). In panels B and D each data point is the mean ± S.E.M. of three to five fibres.

(16–17 fibres) and controls (28 fibres of CCRF-CEM and 41 of MDA-MB-231). For the MDA-MB-231 fibres, only one rat in the single-dose regimen (500 mg/kg) showed a clear effect on the net cell growth compared with controls, while for the five-dose regimen the cell kill increased in a dose-dependent manner (Fig. 2A). In the experiment in which fibres were also retrieved on days 1 and 3 after 375 mg/kg CHS 828, cells in fibres from the single-dose regimen followed a similar growth to those from the control rats (Fig. 2B). The CCRF-CEM cells were more sensitive than the MDA-MB-231 cells, and a dose–response relationship was also seen for the single dose administration with these cells. However, the five-dose regimen still resulted in a larger cell kill, at least for the lower doses (Fig. 2C). In the experiment in which fibres were also retrieved on days 1 and 3, cell survival was similar after both schedules (Fig. 2D).

3.2. Pharmacokinetic analysis

Doses administered on a single occasion ranged from 5.9 to 156 mg. The absorption of CHS 828 was erratic. In the single-dose regimen, for four of five rats with at least one sample containing measurable drug in the range of 13–21 h and one sample in the range of 23–30 h, increasing concentrations were observed (Fig. 3). In order to achieve a successful estimation termination with the covariance step in NONMEM, the two last concentration data points were deleted in an individual rat receiving a single dose of 375 mg/kg who showed a rapid decrease in concentration on the day after administration (Fig. 3). These data points did not influence the choice of best model to fit the data and only marginally influenced the parameter estimates.

The final pharmacokinetic model was a one-compartment linear model with the dissolution and absorption described by a zero-order rate (R1) and a consecutive first-order rate (k_{a1}) input (Figs. 3 and 4, Table 1). OFV dropped 60 units when the fraction absorbed was modelled to decrease with dose, which will here predict a dose-dependent duration of the zero-order rate input. When instead the same function was added on both CL and V the drop in OFV was only 7 units.

Fig. 3. All observed serum concentrations of CHS 828, where zero is the time of the first dose administration (C0). The figure shows smooths (Loess smoother: S-PLUS) of the observations (---), the population predictions (- - -; based on typical population estimates and the model) and the individual predictions (---; based on empirical Bayes estimates and the observed concentrations). For five rats with at least one sample in the range of 13–21 h and one sample in the range of 23–30 h, observations are connected (---). Two observations were omitted in the data analysis (+).
Fraction absorbed \( F \) decreased with increasing dose by a first-order rate (\( k_1 \)). Absorption probably due to coprophagy occurred after a lag time with a first-order rate (\( k_2 \)). The fraction absorbed (\( F_1 \) and \( F_2 \)) decreased exponentially in a dose-dependent manner. Oral clearance (\( \text{CL}(0) \)) increased significantly with cumulative exposure (\( \text{AUC}_0 - \infty \)).

Drug absorption was described by a zero-order (\( k_0 \)) and first-order rate (\( k_2 \)) input. In addition, a fraction (\( F_2 \)) of the dose was modelled to be absorbed after a lag time. The fraction absorbed (\( F_1 \) and \( F_2 \)) decreased exponentially with increasing dose, identical to the function for \( F_1 \). The total fraction absorbed (\( F_2 \)) for the 5.9 mg dose was set as the reference absorbed fraction, i.e. 100%. For the second absorption phase, the data did not support a consecutive zero- and first-order absorption process. Instead the disposition showed flip-flop kinetics as the absorption rate constant, \( k_2 \), was lower (0.0536 h\(^{-1} \)) than the elimination rate constant (ln 2/1.2 = 0.296 h\(^{-1} \) at time zero). The second absorption phase was included after all doses in the predictions. Oral clearance increased significantly from its value at time zero, CL(0), since OFV dropped 27 units when clearance was linearly dependent (by Slope CL) on cumulative exposure, i.e. cumulative AUC (\( \text{AUC}_0 - \infty \)). The model predicted that, on average, clearance increased by 18% on day 5 compared with the first day, in the group receiving 500 mg/kg as a fractionated schedule. A time-dependent \( F_2 \) did not lead to a significant drop in the OFV. IVF was supported for the parameters \( k_1, k_2 \) and CL(0).

Because of the dose-dependent fraction absorbed, the five-dose regimens produced larger AUCs than the single-dose regimens of the same total dose and for the typical individual the model predicted similar AUC values for the three highest single doses (Fig. 5).

### Table 1

<table>
<thead>
<tr>
<th>Estimate</th>
<th>IV (%)</th>
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<tr>
<td>( k_0 ) (mg/h)</td>
<td>2.48 (6.9)</td>
</tr>
<tr>
<td>( k_2 ) (h(^{-1} ))</td>
<td>8.29 (30)</td>
</tr>
<tr>
<td>( F_1 ) &amp; (mg/h)</td>
<td>0.624 (5.7)</td>
</tr>
<tr>
<td>( k_1 ) (mg/h)</td>
<td>0.0067 (6.8)</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>20 (10.28)</td>
</tr>
<tr>
<td>( k_{2,1} ) (h(^{-1} ))</td>
<td>0.0576 (20)</td>
</tr>
<tr>
<td>CL(_{2,1}) (L/h)</td>
<td>0.240 (12)</td>
</tr>
<tr>
<td>Slope(_{2,1}) (L/mg/h)</td>
<td>0.0063 (25)</td>
</tr>
<tr>
<td>( V^* ) (L)</td>
<td>0.811 (8.7)</td>
</tr>
<tr>
<td>( t_{2/1} ) (h)</td>
<td>3.24</td>
</tr>
<tr>
<td>Residual error (%)</td>
<td>38.7 (6.9)</td>
</tr>
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</table>

Drug absorption was described by a zero-order (\( R_1 \)) and a consecutive first-order rate (\( R_2 \)). The fraction absorbed for the dose of 5.9 mg (\( F_{1,\text{max}} \)) decreased with increasing dose by a first-order rate (\( k_2 \)). Absorption probably due to coprophagy occurred after a lag time with a first-order rate (\( k_2 \)). Clearance at baseline (CL(0)) increased linearly (Slope CL) with accumulated AUC, as not supported.

#### 3.3 Toxicity

Weight loss was dose- and schedule-dependent. The two highest doses in each dosing regimen are presented in Fig. 6. At 500 mg/kg, the five-dose regimen produced a more pronounced and prolonged weight loss than the single dose. Haematological toxicity was limited. Leukocyte counts and haemoglobin levels were essentially affected only at the highest dose level, and the five-dose regimen tended to produce the greatest decrease from baseline (Fig. 6). When the dose-dependent pharmacokinetics were accounted for and the individual actual exposure (AUC) was used instead of dose, the schedule-dependent effect was not clear (data not shown). For platelets, no decrease was evident.

#### 3.4 Pharmacokinetic-pharmacodynamic modelling

The growth in unaffected cells and the delayed effect after CHS treatment were well characterized by the model (Figs. 7 and 8, Table 2). Observed intermediate effects were predicted to be due to a too short observation time. A basic
Fig. 6. Effect on haematology and weight after administration of total doses of 375 mg/kg (n = 4 for each schedule) and 500 mg/kg (n = 6 for each schedule) compared with control rats (n = 18), mean ± S.E.M. The observed means are randomly displaced around the true observation day for illustrative purposes.

Fig. 7. Log-transformed observations versus population predictions and individual predictions for the ODs in fibres filled with MDA-MB-231 cells (top) and CCRF-CEM cells (bottom). All predictions are made from individual concentration–time profiles. Lines of identity (—) are included.
Fig. 8. Population predicted profiles (without inter-individual variability; ---), individual predicted profiles (—) and observations (○) of the fibre net growth for MDA-MB-231 (A) and CCRF-CEM (B). All predictions are made from individual concentration-time profiles. The included profiles are from the rats where fibres were retrieved 1, 3 and 5 days after first administration. Each group included two animals (left and right) and the total dose was 375 mg/kg where CHS 828 was administered. Numbers indicate actual observation values for those observations that are above the y-axis range.

$E_{\text{max}}$-model for MDA-MB-231 and a linear model for CCRF-CEM described the concentration–effect relationships the best when the same relationship was used for both schedules. However, when $\text{AUE}_{0-t}$ was substituted for $\text{AUC}_{0-t}$ and separate $\text{AUC}_{\text{thresh}}$ for single and five-daily dosing were applied, the goodness-of-fit improved and the OFV dropped 18 and 10 units for MDA-MB-231 and CCRF-CEM, respectively. Consequently, the final models included linear
concentration–direct effect relationships, but the relationships were different for the two schedules. None of the other investigated concentration–effect relationships could get as good fit to the data as when schedule-dependent AUC_Thresh was used; neither did models where AUC_Thresh was linearly dependent on time of exposure or models where AUC_Thresh was allowed to switch to a lower value at 20, 24 or 30 h.

4. Discussion

As expected, CHS 828 showed a schedule-dependent effect on the net growth of tumour cells after different dose levels, not only for the solid tumour cell line, MDA-MB-231, but also for the more sensitive leukaemia cell line, CCRF-CEM. This is in accordance with a previous study where, at but also for the more sensitive leukaemia cell line, CCRF-CEM, not only for the solid tumour cell line, MDA-MB-231, effect on the net growth of tumour cells after different dose levels was allowed to switch to a lower value at 20, 24 or 30 h.

Parameter estimates (R.S.E.%) and inter-individual variability (IIV) for the final pharmacodynamic model

<table>
<thead>
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<th>MDA-MB-231</th>
<th>CCRF-CEM</th>
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<tr>
<td></td>
<td>Estimate</td>
<td>IIV (%)</td>
</tr>
<tr>
<td>OD_{baseline}</td>
<td>0.459 (2.8)</td>
<td>27 (22)</td>
</tr>
<tr>
<td>( \kappa_{\text{g}} ) (day(^{-1}))</td>
<td>0.178 (3.8)</td>
<td>16 (38)</td>
</tr>
<tr>
<td>Kill</td>
<td>3.27 (1.8)</td>
<td>ns</td>
</tr>
<tr>
<td>AUC_{Thresh} single (mg day/l)</td>
<td>6.35 (&lt;1)</td>
<td>74 (35)</td>
</tr>
<tr>
<td>AUC_{Thresh} five-dose (mg day/l)</td>
<td>2.71 (14)</td>
<td>74 (35)</td>
</tr>
<tr>
<td>Residual error (%)</td>
<td>26.5 (6.7)</td>
<td>ns</td>
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OD at the day of implantation (OD_{baseline}) grew with a first-order rate (\( \kappa_{\text{g}} \)). The rate is affected by Kill when a threshold AUC\(_{\text{Thresh}}\) has been reached (AUC\(_{\text{Thresh}}\); ns: not supported in final models.

a Inter-experimental variability.
b The same IIV was assumed for the single and five-dose schedules.
c 95% confidence intervals were obtained for CCRF-CEM by likelihood profiling (Beal and Sheiner, 1992).

d (approximately 10 h) of the three highest single dose levels. The dose-dependent duration of the input is probably because that after approximately 10 h the drug reaches a part of the intestine where the drug is no longer dissolved and/or absorbed. The mean time for pellets of varying size and density to reach the colon in rats is 5.8–15.6 h (Tuleu et al., 1999). In our study, approximately one third of the systemically available dose was estimated to be absorbed after a lag-time of 20 h after administration, probably as a result of coprophagy. This is a likely explanation to the second absorption phase as the gut transit time in non-fasting rats has been estimated to 21 h (Bungay et al., 1981), together with the fact that CHS 828 has a low solubility, limiting the extent of absorption directly after dose administration. Enterohepatic recycling would not show this pattern as rats lack gall bladder (Strange, 1981). The use of metabolism cages would probably have prevented coprophagy, but only partly (Ebino, 1993). A large IIV in absorption was shown in our study, which also seems to be a problem in patients receiving CHS 828 capsules (Hovstadius et al., 2002).

The half-life of CHS 828 in rats in this study (2.3 h) is similar to that found in patients [2.1 h; (Hovstadius et al., 2002)], but shorter than in our previous study in rats [4.3 h; (Jonsson et al., 2000)]. However, in the latter study, coprophagy was not evident to us and therefore not included in the study, and coprophagy was predicted after all doses. An increasing clearance over time can be due to induction of metabolizing enzymes. CHS 828 is metabolized by CYP 3A4 in man (Hovstadius et al., 2002). Auto-induction phenomena have previously been modelled with a clearance that increase with time of exposure after a lag time (Boddy et al., 1995) and with an inducible enzyme pool that increase with drug concentration (Hassan et al., 1999). However, more data are required before a more sophisticated induction model can be applied to CHS 828.

The pharmacokinetics of CHS 828 appear to be similar in rats and patients, but the toxicity pattern differs.
Thrombocytopenia and gastrointestinal complications were the dose-limiting toxicities of a five-dose schedule of CHS 828 in patients (Hovstadius et al., 2002). However, no reduction in platelet counts was seen in this study in rats. The limited haematological toxicity in rats is in agreement with the higher sensitivity of human lymphocytes (versus rat lymphocytes) to CHS 828 in vitro (Lindhagen et al., 2004). In this study, the dominating toxicity was weight loss; while relatively little haematological toxicity was observed. However, the magnitude and duration of the weight loss was only of importance at the highest dose levels (500 mg/kg). More data, using higher doses if possible, would be required to establish a pharmacokinetic–pharmacodynamic model for evaluation of a “true” schedule-dependent pharmacodynamic effect of haematological toxicity.

The developed pharmacokinetic–pharmacodynamic model resulted in predictions of the cell viability over time that are in agreement with the observations in this study as well as previous observations in vitro (Hassan et al., 2001; Martinsson et al., 2001). The model also clarified that CHS 828 exerts a schedule-dependent pharmacodynamic effect on the investigated tumour cell lines in vivo as described by schedule-dependent AUC_{\text{Thresh}}.

In the pharmacokinetic predictions, a second absorption phase was included after all doses despite that the sampling scheme did not allow this phenomenon to be observed after the fifth dose. A time-dependent change in the extent of the second absorption phase was not found to be significant. Such a change may have however been identifiable as the observed concentrations on the fifth day also included concentrations from the second absorption phase after the fourth dose. Included or not, a smaller second absorption phase than predicted does not affect the conclusion that CHS 828 was schedule-dependent as the AUC from the five-dose schedule would have been smaller with a diminishing second absorption phase. The relatively flat relationship for single doses above 250 mg/kg and AUC (Fig. 5) is not entirely consistent with the observed net growth in Fig. 2. This discrepancy can be explained by (1) a possible slight model-misspecification in the dose-dependency function leading to a too flat relationship, (2) IV in AUC (AUC was generally higher in those rats that showed effect) which will lead to a rapid change in effect around the AUC_{\text{Thresh}} value, and (3) IV and inter-experimental differences in the pharmacodynamics.

Previous knowledge of the observed growth and cell death kinetics was incorporated into the model. If the cell kill mechanism of CHS 828 was known, a more mechanistic concentration-effect model could have been applied, that may allow extrapolation to other schedules than those included in the study. It has been suggested that the schedule dependence is due to two different mechanisms of action of different potencies; the one with the lower potency is independent of exposure time and the more potent mechanism only acts at longer exposure times (Hassan et al., 2001b). However, such models did not give a better fit to the data, either due to limited information in the data and/or due to that the concentrations at the effect site were too low for the less potent mechanism to be significant.

A limitation of the current experimental set-up is the short study period and the full drug effect of the two dosing schedules might not have been observed here. Previous experiments in NYH SCLC xenografts in nude mice (Vig Hjarnaa et al., 1999), using a longer study time, showed that it was the second dose in a once-weekly regimen of CHS 828 that produced the largest reduction in tumour size. In contrast to the current results, a 250 mg/kg per week schedule of CHS 828 was superior to a 50 mg/kg per day schedule in MCF-7 xenografts, already during the first week of treatment (Vig Hjarnaa et al., 1999). In some respects, xenograft models reflect the clinical situation more closely than the hollow fibre model. In xenograft models, tumour blood vessels have time to develop, which may be of importance for drug delivery (Phillips et al., 1998). In addition, prolonged schedules can be studied, that more closely mimic the clinical treatment schedules. In the xenograft studies of Vig Hjarnaa et al. (1999), pharmacokinetics were not investigated. Therefore, it is not known if the fraction absorbed was dose-dependent or, if clearance increased during the study, which was the case here. Moreover, rats and mice may differ in the pharmacokinetics of CHS 828. The diverging results in schedule dependence certainly illustrate the need for studying pharmacokinetic and tumour response in fully understanding this phenomenon.

In this study, the rat hollow fibre model was shown to be suitable for obtaining pharmacokinetic and pharmacodynamic data within the same individual that could be used for population pharmacokinetic–pharmacodynamic modelling of antitumour effects. The combination of the relatively simple tumour model and computer modelling and simulation, could be a helpful tool in drug development. The study also showed that the schedule-dependent antitumour effect of CHS 828 in the rat hollow fibre model is partly due to a dose-dependent fraction absorbed, likely because of low solubility in the gastrointestinal tract, and partly due to schedule dependence in the pharmacodynamics of the drug.

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