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Elin Jonsson · Lena E. Friberg · Mats O. Karlsson
Saadia Bashir Hassan · Agneta Freijs · Klaus Hansen
Rolf Larsson

Determination of drug effect on tumour cells, host animal toxicity and drug pharmacokinetics in a hollow-fibre model in rats

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Abstract *Purpose*: Based on the previously published hollow-fibre assay mainly used for early in vivo anticancer drug screening, we wanted to develop an extended hollow-fibre model in which antitumour activity, haematological toxicity and pharmacokinetics could be studied in the same animal. *Method*: The breast cancer cell lines MDA-MB-231 and MCF-7 were cultured in semipermeable hollow fibres. The fibres were implanted subcutaneously into immunocompetent male Sprague Dawley rats, and the rats were treated with 5-fluorouracil (5-FU, 125 mg/kg), epirubicin (EPI, 10 mg/kg) or cyclophosphamide (CP, 120 mg/kg) intraperitoneally, the new cyanoguanidine CHS 828 (375 mg/kg or 75 mg/kg × 5) orally, or vehicle only. After 6 days the fibres were retrieved and the cell density was evaluated. Haematological parameters were monitored and two to four samples per animal were drawn to determine the pharmacokinetic parameters in NONMEM. *Results*: Drug treatment had generally low effects on the tumour cells. Of the standard drugs (5-FU, EPI and CP), only CP exerted a statistically significant antiproliferative effect. CHS 828 had only a minor effect as a single dose, but divided into five daily doses had a pronounced effect on both cell lines. 5-FU, EPI and CP all caused a marked decrease in leucocytes, platelets and haemoglobin, while CHS 828 did not seem to affect these parameters. The pharmacokinetics of 5-FU and EPI were in accordance with previously established pharmacokinetic models. The pharmacokinetics of CP and CHS 828 were both described by one-compartment models. *Conclusions*: This study illustrates the possibility of measuring antitumour effect, haematological toxicity and pharmacokinetics in the same animal using the hollow-fibre model.

Key words Anticancer drug · In vivo model · CHS 828 · Population pharmacokinetics · NONMEM

E. Jonsson (✉) · S. Bashir Hassan · R. Larsson
Division of Clinical Pharmacology, Akademiska Hospital, SE-751 85 Uppsala, Sweden
e-mail: elin.jonsson@medsci.uu.se
Tel.: +46-18-6114944; Fax: +46-18-519237

L. E. Friberg · M. O. Karlsson · A. Freijs
Division of Biopharmaceutics and Pharmacokinetics, BMC, SE-751 23 Uppsala, Sweden

K. Hansen
Leo Pharmaceuticals, Industriparken 55, DK-2750 Ballerup, Denmark

Introduction

For testing the antitumour properties of a new drug in vivo, there are a variety of animal tumour model systems available, ranging from murine transplantable tumours to xenograft models in which human tumour cell lines are cultivated in nude mice [1]. Since xenograft models are relatively laborious and expensive, there is a need for simpler animal models still using tumour cells of human origin. In 1995, an alternative in vivo model was presented, based on the cultivation of tumour cells in semipermeable hollow fibres [2]. The original hollow-fibre model is now used routinely at the National Cancer Institute (NCI) for in vivo drug screening [3]. Cell lines from the NCI drug screening cell-line panel are cultured in semipermeable hollow fibres, and nude mice are used as host animals. The results from the hollow-fibre model are used to guide the prioritization of compounds for xenograft testing, and also to aid the selection of suitable cell lines to use in the following xenograft studies.

Traditionally, drug toxicity is studied separately in non-tumour-bearing animals from different species, with the aim of finding tolerable doses and characterizing drug-specific toxicity [4]. The results from the toxicity studies form the basis for dose selection both for further in vivo studies and for the first clinical trials.

The preclinical pharmacokinetic properties are generally investigated in separate pharmacokinetic studies or as a part of toxicity studies. In clinical trials the use of sparse blood sampling and the development of population pharmacokinetic models are well accepted. Despite

their potential [5], preclinical data are rarely used and for anticancer drugs there are just a few examples of preclinical population models in the literature [6–8]. Even if the interindividual variability is less in laboratory animals than in patients, it is not negligible. Characterization of interindividual variability in the pharmacokinetics leads to better pharmacokinetic-pharmacodynamic relationships, even with just a couple of concentration samples per individual [9] and established relationships could provide valuable information to improve clinical trials.

We wanted to develop an animal model in which pharmacokinetics, tumour effects and haematological toxicity of anticancer drugs could be studied in the same animal. The hollow-fibre method seemed to be an attractive effect model and we wanted to evaluate it for our purpose in immunocompetent rats. This new hollow-fibre method is described here and was used in an investigation of three cytotoxic drugs commonly used in breast cancer, 5-fluorouracil (5-FU), epirubicin (EPI) and cyclophosphamide (CP) (combined in the FEC regimen) and one novel substance with antitumour properties (CHS 828).

Materials and methods

Drugs

5-FU (Flurablastin, 50 mg/ml; Pharmacia & Upjohn), EPI (Farmorubicin; Pharmacia & Upjohn) dissolved in sterile water to a concentration of 2 mg/ml and CP (Sendoxan; ASTA Medica) dissolved in sterile water to a concentration of 20 mg/ml were prepared in syringes by the local pharmacy. Corresponding volumes of sterile normal saline were used for the control animals. The syringes were used within 24 h.

CHS 828 (*N*-(4-chlorophenoxyhexyl)-*N*′-cyano-*N*″-4-pyridylguanidine) was provided by Leo Pharmaceutical Products (Ballerup, Denmark) and formulated at 10 or 50 mg/ml as a suspension with methyl cellulose (2% or 10%) in water. The more concentrated suspension was used for the single dose administration. The suspensions were ultrasonicated for 30–60 min and kept refrigerated in dark glass bottles for a maximum of 1 week. Before each administration, they were mixed thoroughly with a magnetic stirrer. Water with 2% or 10% methyl cellulose was administered to the control rats in experiments involving CHS 828.

Cell lines

The human breast cancer cell lines MDA-MB-231 and MCF-7 were kind gifts from Jonas Bergh of the Department of Oncology, Uppsala University [10, 11]. The MDA-MB-231 cells were maintained in RPMI-1640 culture medium and the MCF-7 cells in minimum essential medium, both media supplemented with 10% fetal calf serum, glutamine and streptomycin/penicillin (all from Sigma, St Louis, Mo.). The cells were passaged twice a week and harvested with trypsin/EDTA (Biochrome, Berlin, Germany).

Hollow fibres

The hollow-fibre procedure was modified from that described by Hollingshead et al. [2]. Polyvinylidene fluoride (PVDF) hollow fibres (500 kDa molecular weight cut-off, 1 mm inner diameter; Spectrum, Laguna Hills, Calif.) were conditioned by washing and incubation with 70% ethanol for a minimum of 72 h, followed by washing in Millipore water and autoclaving.

The fibres were cut into 100-mm lengths, washed with complete RPMI medium and emptied. Cell suspension (2×10^6 cells/ml) was flushed into the fibre with a 0.90-mm needle and a 1-ml syringe, and the ends of the fibre were sealed with a smooth-jawed needle holder heated in a flame. Individual implants were made by making double heat-seals in the fibre at 20-mm intervals and cutting between them. The fibres were transferred to petri dishes with complete RPMI medium and incubated for 48 h prior to implantation into the rats.

To investigate the variability in the fibre implant length after the manual fibre preparation procedure, the actual length of every fibre was measured after retrieval in some of the experiments. The coefficient of variation of the fibre lengths within each experiment was no higher than 5% (data not shown).

Animal procedure

The study was approved by the Animal Ethics Committee in Uppsala (No. C220/96 and C67/99). Anaesthesia was induced in male Sprague Dawley rats (weight 295 ± 7 g; Charles River, Uppsala, Sweden) by inhalation of enflurane (2.5%, Efrane; Abbot) mixed with nitrous oxide (1.5 l/min) and oxygen (1.5 l/min). After shaving, two parallel rows of eight small skin incisions were made with sharp scissors on the back of the animal. A 100-mm piece of 2–0 silk suture was tied around one end of each fibre implant. The fibre was inserted subcutaneously between two skin incisions in the dorsocaudal direction with the help of the suture and a trocar. Eight fibres were implanted on each animal, and the incisions were closed with tissue glue (Indermil; Kendall Medical, Sweden) and skin staples. The suture was cut short and adhered to the tissue glue.

Each animal carried three fibres with each of the two breast cancer cell lines and one fibre with medium only. In every experiment, six rats were randomized into three groups, making it possible to study two different drug regimens together with a control in each experiment. Every drug was run in two or three independent experiments.

Drug administration was started the day after surgery. 5-FU (125 mg/kg), EPI (10 mg/kg) and CP (120 mg/kg) were given as single intraperitoneal injections and the doses were close to the maximum tolerated doses according to previous studies. CHS 828 was administered orally as a single dose of 375 mg/kg or as 75 mg/kg for five consecutive days. This dose was chosen based on previous hollow-fibre studies in which it had shown antitumour activity in other cell types without significant host-animal toxicity (data not shown).

The fibres were retrieved 5 days after the first administration from anaesthetized animals by opening the rostral incisions and pulling the suture. After retrieval the incisions were closed again with tissue glue. The suture was removed from each fibre, and the fibres were collected in six-well plates filled with culture medium and kept for a maximum of 4 h at 37 °C until cell staining.

Living cell density

The cell density was evaluated by staining with MTT [(3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma], which is converted by metabolically active cells to blue formazan crystals that are trapped in the cells.

The retrieved fibres were washed in trypsin for 5 min to release potential host cell growth outside the fibre. The fibres were then incubated in six-well plates with 3 ml complete medium and 200 µl MTT stock solution (5 mg/ml in phosphate-buffered saline; Sigma) at 37 °C for 4 h. The staining was stopped and the fibres were washed overnight by incubation at 4 °C in a buffer containing phosphate-buffered saline with 2.5% of a protamine sulphate stock solution (1%; Sigma). Each fibre was rubbed carefully and cut in half. A separate investigation with medium-filled fibres retrieved from untreated rats indicated that the washing step in trypsin and

the removal of the suture before MTT staining could be omitted without affecting the performance of the method (data not shown).

The fibres were placed into 24-well plates (each fibre in a separate well), where all fibres from one experiment were allowed to dry until the day of analysis. The formazan produced in the MTT staining reaction was extracted with 250 µl dimethylsulphoxide (DMSO; Sigma) per well for 4 h. The extract was transferred in 150-µl aliquots to flat bottomed 96-well plates and the absorbance was read at 570 nm in a plate reader (Dynatec Laboratories, UK). Because some wells showed out-of-range absorbance (OD > 2.5), the extracts were diluted with DMSO (75 µl + 75 µl), and this diluted data set was used in the calculations. The absorbance value from blank wells with DMSO only was subtracted from each reading.

The cell density was determined for triplicate fibre samples from in vitro incubation on the filling day, implantation day and day of retrieval to assess the in vitro growth of the cells. To be able to take into account possible differences in cell density on implantation day when assessing differences in drug effects on cell growth, an analysis was performed using extended least squares within NONMEM [12] with the following model: absorbance = baseline + growth. All fibre absorbance values from implantation day (baseline) and from the animals on retrieval day for a certain drug and cell line, and corresponding data from fibres from vehicle-treated animals were included in the analysis. Different growths were considered for the fibres from drug-treated and vehicle-treated rats as well as different baseline and growths for the different experiments. An additive and a proportional error model were tried. Comparison between hierarchical models was performed using the NONMEM objective function value (two times the log likelihood value) as differences in this value show approximately a χ^2 distribution. Therefore, the likelihood ratio test with degrees of freedom given by the difference in number of parameters for the two models can be used. In this modelling a significance level of $P < 0.05$ was used, which for a one-parameter difference is 3.84.

The cell density in the fibres from the animals was expressed as net growth, defined as:

$$\frac{(\text{absorbance on retrieval day} - \text{mean absorbance on implantation day})}{\text{mean absorbance on implantation day}} \times 100\%$$

When no significant interexperimental differences were detected in the NONMEM analysis, absorbance data from repeated experiments were pooled. Pooling the data helps in the interpretation of the results and increases the power to detect differences.

Haematological toxicity

Blood (250 µl) for haematological toxicity determination was collected in the morning 1 day prior to implantation (baseline) and on days 4, 6, 8 and 11 after drug administration. The blood was sampled from the hind paw vein after the rats had been placed on a heating pad for at least 15 min, and collected into EDTA-prepared Microtainer tubes (Becton Dickinson, N.J.). The numbers of leucocytes, platelets and haemoglobin were determined in a Coulter Counter (MDII series; Coulter Electronics, Luton, UK), and are presented as percent of the baseline value. The animals were weighed every day of handling, and the weight gain is presented as percent of the baseline weight.

Pharmacokinetic sampling

To determine the plasma concentration of 5-FU, EPI and 4-hydroxy-cyclophosphamide (4-OHCP, the active metabolite of CP), two blood samples (500 µl) were collected from the hind paw in cold Microtainer tubes. The sampling interval after dosing was 15–150 min for 5-FU and 15–240 min for EPI and 4-OHCP. The samples were chilled on ice for a couple of minutes before centrifuging at 4°C for 5 min at 7200 *g* (5-FU and EPI) or 6 min at 2600 *g* (4-OHCP). For 5-FU and EPI the plasma was then frozen on dry ice. For 4-OHCP, 150 µl plasma was mixed with 300 µl cold acetonitrile and centrifuged for 6 min at 2600 *g*. The supernatants (300 µl) were directly frozen on dry ice. All samples were stored at −80°C until analysis.

From the CHS 828-treated rats, four blood samples (250 µl; sampling range 10–480 min), were collected in Eppendorf tubes and kept cold for 30–60 min. The samples were then centrifuged for 10 min at 3500 *g*. The serum was transferred to new Eppendorf tubes and kept at −80°C until analysis.

Chemical analysis

5-FU concentrations were determined by an HPLC method with UV detection after an extraction step with ethyl acetate. 5-Bromouracil was used as internal standard. EPI was quantified by an HPLC method with fluorescence detection after a solid phase extraction step on Sep-Pak cartridges (Waters Corporation, Milford, Mass.). The same procedures as in an earlier analysis performed by us were applied [6]. For 4-OHCP, a reversed-phase HPLC method with UV detection was used after a derivatization step with 2,4-dinitrophenylhydrazine (Merck, Darmstadt, Germany). The method has been described in detail elsewhere [13]. However, we validated the method for 150 µl rat plasma (300 µl supernatant) in the range of 0.034–10 mg/l. The within-run and between-run precision at three concentrations were less than 5% and 9%, respectively. The accuracy was between 95% and 106%. At the limit of quantification, 88% was found and the coefficient of variation was 11%. Samples predicted to be above the validated range of the analytical methods were diluted with plasma/supernatant prior to work-up.

Determination of CHS 828 was performed by Leo Pharmaceuticals using an HPLC method with UV detection at 277 nm. Prior to work-up, 50 µl rat serum was mixed with 950 µl blank serum. EO 859-000 (Leo Pharmaceuticals) was added to each sample as internal standard, followed by the addition of ammonia and subsequent extraction with *tert*-butyl methyl ether. The organic layer was back-extracted with an aqueous solution of phosphoric acid, and ammonia was added before HPLC analysis. A reversed-phase C-18, 3.5 µm, 100 × 2.1 mm column and a C-18, 3.5 µm, 10 × 2.1 mm precolumn (Waters) were used. Elution was performed with a linear gradient using mixtures of acetonitrile, aqueous phosphate buffer and dimethyl-octyl amine as eluents. In each run, quality control samples in duplicate at three levels covering the analytical range 2.5–1000 ng/ml using 1 ml serum were included. The in-process precision ranged from 8% to 15%, while the accuracy ranged from 91% to 97%.

Pharmacokinetic data analysis

The pharmacokinetic parameters of 5-FU and EPI were determined in NONMEM by Bayesian analysis with pharmacokinetic models derived from the same type of rats under similar conditions [6]. A one-compartment model with capacity-limited elimination with instant and complete absorption was used in the 5-FU analysis and a three-compartment model with first-order absorption and a bioavailability of 71% was used to describe the EPI data (Table 1). To determine whether the pharmacokinetics in the 5-FU- and EPI-treated rats in this study differed from those in the rats from which our population models were derived, the magnitudes of the residual error were estimated in the hollow fibre-implanted rats, keeping the other model parameters fixed. The magnitudes of the residual error were then compared with the magnitudes of the residual error in the original model.

To analyse the population pharmacokinetics of 4-OHCP and CHS 828, the data from the present study were pooled with data from additional rats from other 4-OHCP ($n = 34$) and CHS 828 ($n = 12$) studies. These studies were performed under similar conditions in our laboratory, but for CHS 828 the sampling time was extended up to 30 h. The first-order (FO) method within NONMEM was used and interindividual variability was allowed on the parameters. Additive and proportional error models were tried.

Table 1 Pharmacokinetic parameters used for 5-FU and EPI in the Bayesian analysis, and estimated parameters for 4-OHCP and CHS 828. Shown are the population mean parameters and, if included in the models, interindividual variability (CV%)

	5-FU	EPI	4-OHCP	CHS 828
CL (l/h)	–[a]	1.1 (40%)	2.0[b]	0.65[b]
Vss (l)	0.24	54 (V1 39%)	1.8[b] (22%)	4.0
Ka (h^{-1})	>1000	2.4	11	2.2[c]

[a] Michaelis Menten kinetics: Vmax = 103 mg/(l × h), Km = 16 mg/l (CV = 16% for both parameters)
[b] Apparent clearance and volume, not corrected for bioavailability or fraction metabolized
[c] Preceded by a zero order rate constant of 11 mg/h

To discriminate between hierarchical models, a significance level of $P < 0.001$ was used in this analysis, which for a one-parameter difference corresponds to a decrease in the objective function of 10.83. In addition, the program Xpose was used for model diagnostics [14].

Results

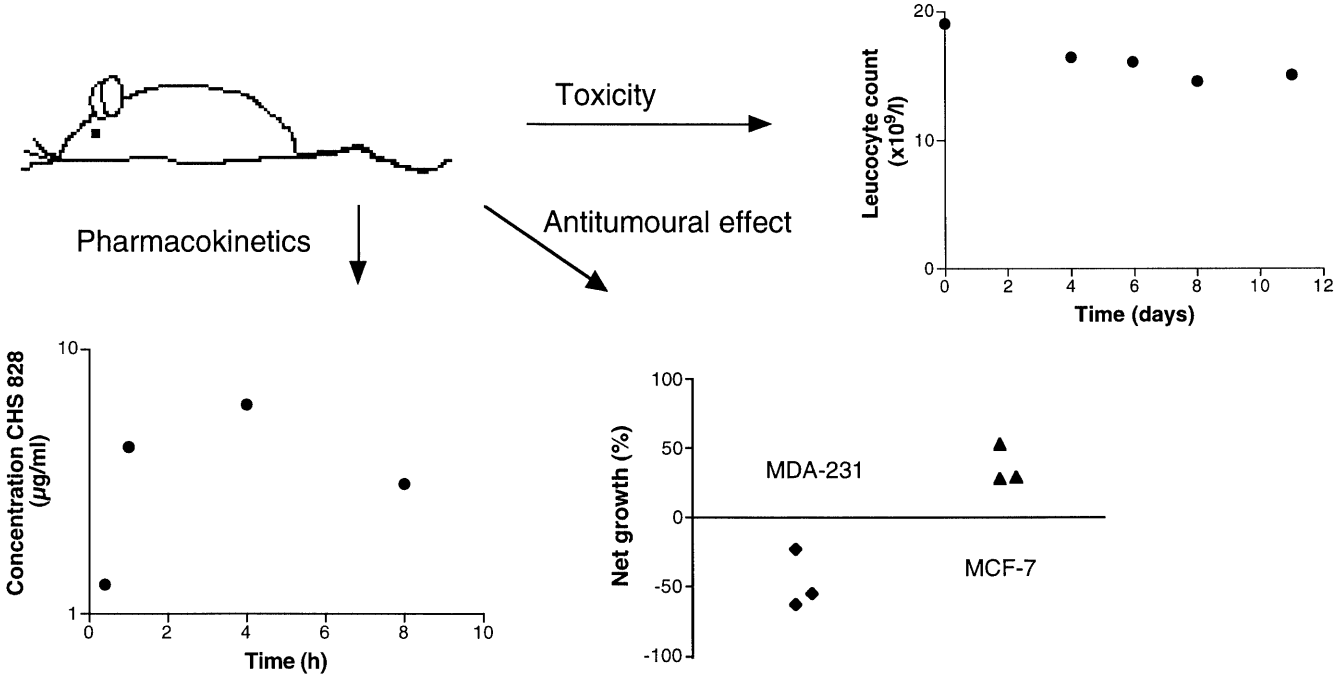
Figure 1 illustrates the possibility of obtaining different data from one single animal. Data from a typical rat receiving 75 mg/kg CHS 828 for 5 days is shown. In this rat, four samples were taken for the determination of the serum level of CHS 828 and five samples for haematology monitoring, exemplified by the leucocyte count. The antitumour effect was determined in three MDA-MB-231-containing fibres and three MCF-7-containing fibres.

The effect of drug treatment in vivo on the breast cancer cell lines was generally low. No significant effect of 5-FU or EPI on the growth of either cell line was found when all data (except for data from one experiment on MCF-7 cells where the cells died in the control cultures) were pooled in the NONMEM analysis. The effect of CP was low, but overall statistically significant, in both cell lines. When given as a single dose, CHS 828 exerted a low but statistically significant effect on MDA-MB-231 cells but not on MCF-7 cells. The effects of CHS 828 divided into five daily doses on the growth of both cell lines were pronounced.

A lower baseline in one of the three experiments evaluating the effects of CP on MDA-MB-231 cells and a lower control cell growth in one of the two experiments evaluating the effects of CHS 828 on MCF-7 cells were the only significant interexperimental variability found in the NONMEM analysis. These two experiments were excluded when the data from repeated experiments ($n = 2$–3) were pooled and the effect of drug treatment on the fibres is presented as net growth (percent) in Fig. 2. The antiproliferative effect of CHS 828 given as a 5-day schedule were apparent in MCF-7 cells as a decrease in net growth, while in the case of MDA-MB-231 cells a negative net growth indicated a cell killing effect (Fig. 2).

The rats receiving 5-FU, EPI and CP showed a pronounced decrease in leucocytes with an average nadir value of 44%, 21% and 8.5% of baseline, respectively (Fig. 3a). The time-course of thrombocytopenia showed a similar pattern but the effect in the 5-FU-treated rats was more pronounced than for leucocytes with a nadir of 10% of baseline (Fig. 3b). Haemoglobin had

Fig. 1 Example of data obtained from one rat receiving CHS 828 75 mg/kg orally for 5 days. Toxicity is exemplified by five measurements of leucocyte count, antitumour effect by measurement of net growth of three fibres containing MDA-MB-231 cells and three fibres containing MCF-7 cells, and pharmacokinetics by four measurements of the serum level of CHS 828



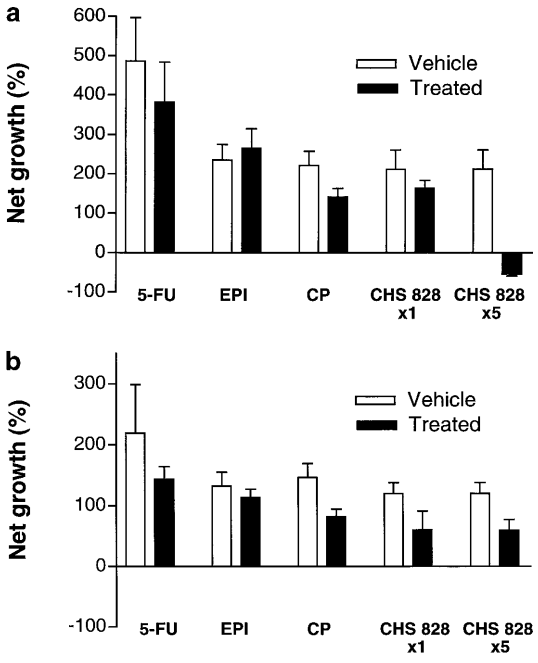


Fig. 2a,b Effects of different drugs on the breast cancer cell lines MDA-MB-231 (**a**) and MCF-7 (**b**) in the hollow-fibre system in vivo. Data from one to three experiments are presented as percent net growth ± SEM

decreased to about 72–76% of baseline by day 11 in the 5-FU-, EPI- and CP-treated rats (Fig. 3c). However, the haematological parameters of the rats receiving either regimen of CHS 828 were practically unaffected and indeed showed rather an increase than a decrease compared with the control rats. The weight loss was greatest in the EPI- and CP-treated rats while the weight gain in the 5-FU- and CHS 828-treated rats was reduced compared with the vehicle-treated rats up to day 6 after treatment (Fig. 3d).

The magnitudes of the residual error in the 5-FU- and EPI-treated rats were lower or within the confidence limits of the original pharmacokinetic models and therefore the Bayesian analysis performed was concluded to be valid for these rats. The pharmacokinetics of 4-OHCP was best described by a one-compartment model with first-order absorption (Table 1, Fig. 4a), and the residual error was estimated as 16%. The disposition of CHS 828 was characterized by a one-compartment model with the dissolution and absorption of drug described by a consecutive zero-order rate and a first-order rate input (Table 1, Fig. 4b). For CHS 828, log-transformed concentration data were used. The data did not contain enough information to separately identify interindividual and residual variability and the combined variability component was estimated as 82%. The calculated half-lives of 4-OHCP and CHS 828 were 37 min and 4.3 h, respectively.

Discussion

We performed the hollow-fibre experiments in immunocompetent rats. The use of non-immunocompromised animals reduces the costs, makes the handling easier and allows determination of haematological toxicity. Rats are preferable to mice since they can withstand serial bleedings needed for the characteriza-

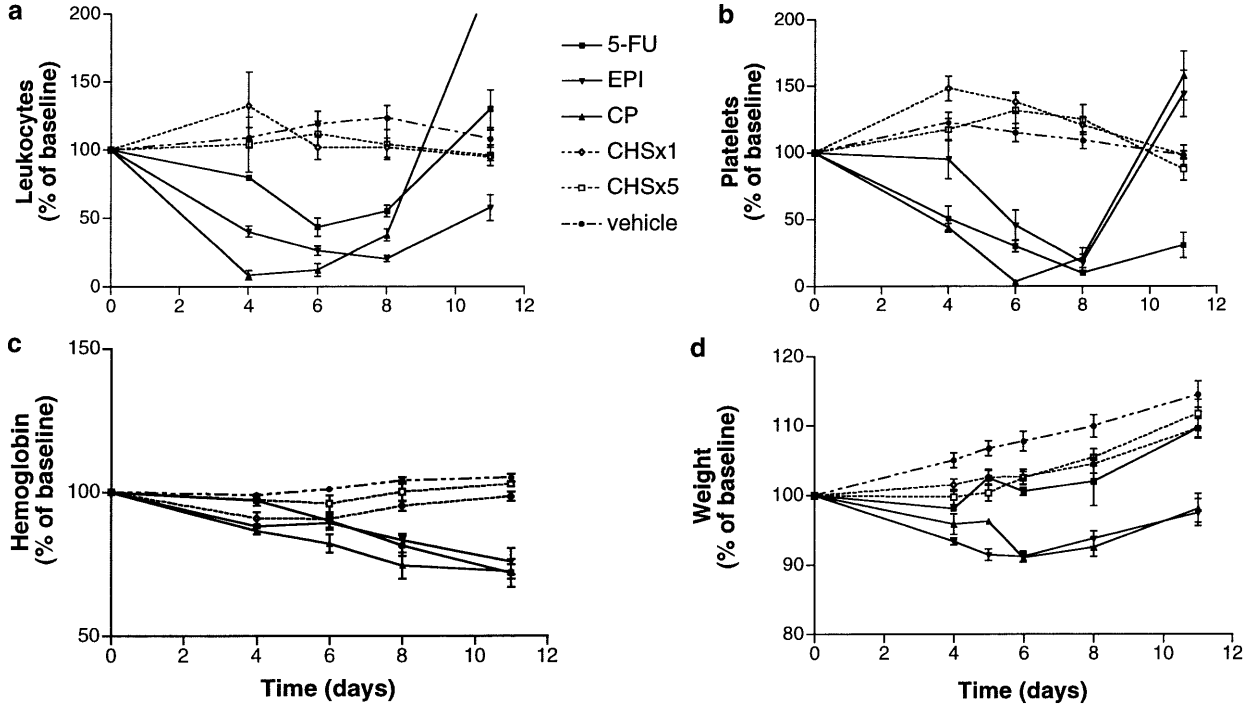


Fig. 3a–d Effect of drug treatment on (**a**) leucocytes, (**b**) platelets, (**c**) haemoglobin and (**d**) weight of the rats. The data are presented as percent of the baseline values; *error bars* indicate SEM ($n = 4$–9)

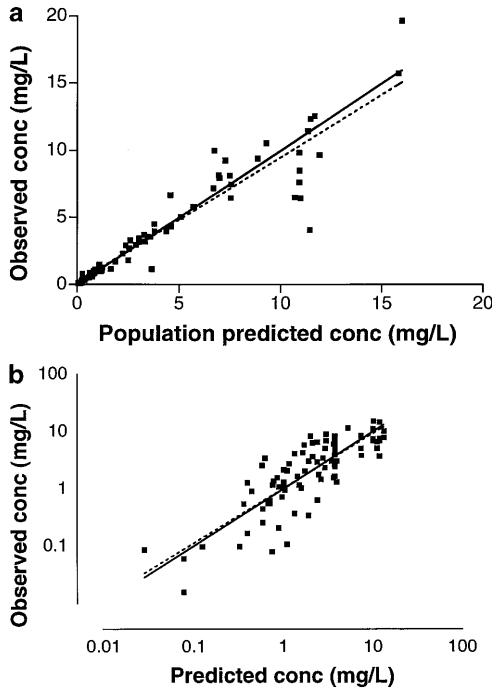


Fig. 4a,b Goodness of fit plots from the pharmacokinetic analyses of (**a**) 4-OHCP and (**b**) CHS 828 (— line of identity, - - - regression line)

tion of pharmacokinetics and haematological toxicity simultaneously. Rats can also be host to more hollow fibres at the same site, i.e. more effect measurements can be performed in the same individual. Since eight fibres can be implanted into each rat, it is possible not only to study multiple samples of two cell types, but also to add permanent controls, e.g. blank fibres containing medium only or positive controls with known sensitive cell lines. Implanting the fibres subcutaneously and using intraperitoneal or oral dosing avoids the semi-in vitro situation that arises if drug is administered to the fibre implantation site. We chose to use a time frame of 6 days in the rat, comparable to the NCI experiments [2]. In this set-up with six animals, one experiment, in which the effects of two different drugs or dose levels on two different cell types are investigated, can easily be handled by two people every 2 weeks.

The FEC regimen, consisting of 5-FU, EPI and CP administered together on one occasion, is widely used in the treatment of metastatic breast cancer, and may produce clinical response rates of up to 90% [15]. In the present hollow-fibre study, of these drugs only CP had a significant effect on the tumour growth in vivo despite the relatively high drug doses administered. The reasons for this might be that the MDA-MB-231 and MCF-7 cell lines are relatively resistant, that a combination of two or three of the drugs is necessary to produce an effect or that drug delivery is dependent on blood vessel development. Low effects of standard chemotherapeutic agents on MDA-MB-231 cells cultured in subcutaneous fibres in the original hollow-fibre model have also been reported [2].

CHS 828 is a cyanoguanidine presently under study for its antitumour properties and has shown promising activity both in vitro and in other in vivo systems [16]. Studies in rodent tumours as well as in xenografts of the lung cancer cell line NYH and the breast cancer cell line MCF-7 have revealed a high activity of oral CHS 828 [16], and this promising finding led us to test CHS 828 in our hollow-fibre assay. Cytotoxic effects of CHS 828 were also shown in the present study. When comparing the results for CHS 828 with those for the standard drugs, it must be borne in mind that the dose used for CHS 828 was not significantly toxic to the animals, while the other drugs were used close to their MTD. Despite this, CHS 828 exerted a higher antitumour activity.

In the present study, CHS 828 was administered as one dose (375 mg/kg) or divided into five daily doses (5×75 mg/kg) with the aim of mimicking the two dosing schedules now in use in the first clinical trials with the compound. The finding that CHS 828 had higher antitumour activity, without an obvious difference in toxicity, in the 5-day dosing schedule compared to the 1-day dosing schedule is interesting. In the pharmacokinetic analysis no dose dependencies were found, and the total exposure (AUC) of CHS 828 seemed, therefore, to be the same for both schedules. The results from this 6-day study could be compared with previous long-term studies in xenografts of NYH and MCF-7 cells, in which once-weekly dosing of CHS 828 has shown an equal or better effect than a once- daily dosing [16]. The reasons for this discrepancy between different studies, and the pharmacokinetic and/or pharmacodynamic reasons underlying the schedule dependency are still not known. Studies are currently underway to investigate this further, and the results could have an impact on the design of new clinical schedules. Characterization of schedule dependency is desirable for many drugs, since the effect and dose-limiting toxicity can have different schedule-dependent patterns and the establishment of pharmacokinetic-pharmacodynamic relationships could help in finding a schedule with an optimal therapeutic index. To do this it is important to study the effect and toxicity relationships within the same species and, preferably, within the same animal. Our extended in vivo hollow-fibre model seems to have the potential for use in the study of the schedule dependency of anticancer drugs in terms of their antitumour effect, toxicity and pharmacokinetics.

Others have studied drug delivery and blood supply to subcutaneous hollow fibres, and have concluded that the hollow-fibre model in short-term mode is a relatively resistant model because of poor drug delivery to the cells [17]. Blood vessels need time to develop, and thus the drug in these experiments must be able to reach the fibre by, for example, passive diffusion. This may be one explanation for the poor effect obtained with most drugs in this study. However, drug delivery problems are

probably also relevant to the actual clinical situation, since tumours often have a poor blood supply and there is a need for new drugs with good delivery properties. CHS 828 showed significant antitumour activity despite the low blood supply, which is interesting.

Three different approaches can be used in the determination of individual pharmacokinetic parameters in population analysis: (1) only data from the presented study are used to develop the pharmacokinetic model, (2) data from other studies are mixed with those from the presented study for stabilization or (3) a Bayesian analysis of the presented data is performed with a previously established population model. We used the second and third of these approaches in our study. By using a population approach a full picture of the pharmacokinetic-pharmacodynamic relationships could be obtained, both regarding the effect and the toxicity. Characterization of pharmacodynamic interactions is another potential use of modelling and could be especially useful in the field of cancer treatment where several drugs are often combined to increase the therapeutic efficiency.

The advantages of studying pharmacokinetics in the same animals as those used for toxicity evaluation have been previously discussed [18]. First, the number of animals and workload involved are decreased, second, the pharmacokinetics can be used to explain extreme findings in an animal and, third, individually based concentration/effect relationships can be derived. Such relationships may aid in the development of human dosing regimens [19] and it has been shown that individual kinetic parameters reduce the variability in EC_{50} in rats [20]. Thus, properly accounting for individual pharmacokinetic differences can impact on the determination of pharmacodynamic relationships. For this reason others have also used the technique of obtaining pharmacokinetic and pharmacodynamic data in the same animals [21, 22]. In addition, surgical procedures might affect the drug disposition [23] leading to differences between rats with and those without fibres.

To summarize, we showed that the in vivo hollow-fibre method makes it possible to study the effects of anticancer drugs in several cell lines, haematological toxicity and pharmacokinetics within the same animal. Apart from establishing pharmacokinetic-pharmacodynamic relationships, the model could also be used for other related problems. Studies ongoing in our laboratory include an evaluation of culturing primary human tumour cells from patients in the hollow fibres, studies of schedule dependence and studies of the time-course of drug effects.

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