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Fluorouracil Catabolism in the Combination Treatment of Cyclophosphamide, Methotrexate and Fluorouracil

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The CMF-regimen is amongst the most effective chemotherapeutic approaches in the treatment of breast cancer. It is generally accepted that the efficacy of the combination of the three agents used in the regimen, i.e., cyclophosphamide (CY), methotrexate (MTX) and fluorouracil (FUra), is based on interactions between the drugs at the intratumoral level. In WAG/Rij rats we previously demonstrated that change of FUra clearance at the first day of the CMF-regimen occurs owing to concomitant CY+MTX. In the present study clearance of FUra and the first product of FUra catabolism, $FUraH_2$, were monitored at day 1 and day 8 of the regimen upon treatment with single agent FUra (F), MTX+FUra (MF), CY+FUra (CF), and CY+MTX+FUra (CMF). At the first day of treatment, FUra and $FUraH_2$ systemic exposure was demonstrated to be increased in CMF-treated rats owing to concomitant CY+MTX. At the eighth day of treatment it was found that repeated CY administration during the previous seven days in CF-treated rats resulted in increased FUra and $FUraH_2$ systemic exposure and therefore increased the dose of FUra artificially. It is concluded that altered FUra clearance owing to extratumoral interactions by concomitant CY and MTX contributes to the efficacy of the CMF-regimen.

Key words: Breast cancer — Fluorouracil — CMF regimen — Catabolic breakdown

Fluorouracil (FUra) is a fluorinated pyrimidine which has been used in the treatment of gastrointestinal, breast and ovarian cancers since its introduction into clinical practice. In attempts to improve the efficacy of FUra, studies which combine FUra with other antitumor agents have been explored, such as the CMF regimen of cyclophosphamide (CY)+methotrexate (MTX)+FUra in the treatment of breast cancer.[1–5] Up to the present, the CMF regimen has been applied with variable success rates[5–10]; the most profound effect was demonstrated in node positive, premenopausal breast cancer.

Although studies concerning fluoropyrimidine pharmacokinetics in single agent sessions have been reported extensively,[11–13] data on FUra pharmacokinetics associated with administration of CY and MTX in the CMF regimen are limited.[14–17] Patient studies do not allow demonstration of interactions of the drugs at the pharmacokinetic level; this was systematically investigated by us in a standardized animal model.[15–17] Mutual interactions between CY, MTX and FUra at the pharmacokinetic level have been profiled for all these drugs after stepwise combinations at the first day of the regimen: CY (C) vs. MTX (M) vs. FUra (F) vs. CY+MTX (CM) vs. MTX+FUra (MF) vs. CY+FUra (CF) and CY+MTX+FUra (CMF). In the present study we extended our observations by including data on FUra and 5,6-dihydro-5-fluorouracil ($FUraH_2$) levels at two different days of the CMF regimen in F-, MF-, CF- and CMF-treated rats. The drugs of interest are all combined at days 1 and 8 in the clinically applied regimen. At the other days, i.e. days 2–7 and 9–14, CY only is administered in the treatment modalities CF and CMF. The present study was intended to obtain a deeper understanding of the acute interactions at day 1 as well as the possible consequences of intoxication or induction of enzyme systems involved in drug clearance processes at day 8.

At the first day of the CMF regimen for instance, cytotoxic metabolites of CY are generated upon administration. They are immediately formed and might influence the clearance processes of MTX and/or FUra. At the eighth day, however, the inducing effects of the only agent which is administered daily, i.e. CY, might lead to induction or inhibition of enzyme systems which are involved in clearance processes of the agents used in the CMF regimen. Doses of CY, MTX and FUra administered to animals included in the present study were chosen by a new approach: doses were selected to give plasma levels close to those found in patients treated according to the CMF regimen.[14–17] This procedure should allow comparisons with clinical data concerning the CMF regimen.

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MATERIALS AND METHODS

Drugs FUra was supplied by Hoffmann-La Roche (Basle, Switzerland), MTX by Pharmachemie B.V. (Haarlem, The Netherlands) and CY by ASTA (Bielefeld,Germany).

Animals Female WAG/Rij rats (180–200 g) were cannulated under light ether anesthesia one day before treatment with FUra alone (F), FUra in combination with CY (CF), FUra in combination with methotrexate (MF) and FUra in combination with MTX and CY (CMF). From cannulation until the end of the kinetic experiments the rats were starved, while water was supplied *ad libitum*. Flexible cannulas, filled with a saline solution containing heparin at the end of the cannula, were inserted into the carotid arteries. During the experiments the rats were kept in cages in which free movement was possible despite the cannulation. Each cage contained one rat; the cages were placed in a temperature-controlled room at 20°C, illuminated from 7 AM till 7 PM.

Drug treatment Drug treatment started at 9 AM. FUra and combinations of FUra with CY and MTX were administered to at least 15 animals per group. MTX (2 mg/kg) and FUra (20 mg/kg) were administered systemically; CY (7 mg/kg) was administered orally by stomach tube. CY was administered daily during 14 days, while MTX and FUra were administered at days 1 and 8 only. That schedule is in accordance with the CMF-regimen applied in clinical sessions, including oral CY. The doses were chosen in such a way that blood plasma concentrations generated were comparable to those observed in patients.[14–18] The sequence of administration was always: 1) CY, 2) MTX and 3) FUra in cases of concomitant drug administration. The four treatment modalities, F, CF, MF and CMF, were equally spread over the whole period of the experiment. Microvolumes (250 μl) of blood were collected at appropriate time intervals for pharmacokinetic analysis. Blood samples were centrifuged and plasma was stored at -35°C until analysis.

Determination of FUra and $FUraH_2$ FUra and its primary product of catabolism $FUraH_2$ were determined simultaneously by capillary gas chromatography (GC) using 100 μl of rat blood plasma only.[18] Elution of compounds of interest was carried out on support-coated open tubular OV-275 columns with 5-chlorouracil as an internal standard. A two-step liquid-liquid extraction was performed with chloroform (clean-up) and ethyl acetate, respectively. The ethyl acetate layer was removed and evaporated under a gentle stream of nitrogen. Special care was taken to prevent degradation of $FUraH_2$: plasma was adjusted to pH 3.5 and extraction was performed on ice.[19] Using electron capture detection (ECD), the limit of detection for both FUra and $FUraH_2$ was 10 ng/ml.

Data analysis Comparisons of plasma concentration per time-point were carried out by one-way analysis of variance (ANOVA) using Scheffé's procedure for multiple comparisons. In order to compare concentrations of all treated groups over the whole set of time points, i.e. the plasma concentration-time curves, the Friedmann two-way analysis of variance test was used. For all tests $P<0.05$ was taken as the criterion of significance. Pharmacokinetic calculations were carried out by non-compartmental analysis. The elimination half-life ($t_{1/2,z}$) was calculated by linear regression analysis of the terminal part of the plasma concentration-time curve. The area under the plasma concentration-time curve (AUC) was calculated by use of the trapezoidal rule with extrapolation to infinity. Total body clearance (CL_{TB}) was calculated as dose/AUC. Statistical analysis of pharmacokinetic data was focused on comparing data over the four groups using ANOVA with Scheffé's procedure for multiple comparisons. In some experiments to compare F+CF with MF+CMF and F+MF with CF+CMF, Student's two-sample t test was applied. Pharmacokinetic data of days 1 and 8 within one treatment group were also evaluated by using Student's two-sample t test. For all tests, $P<0.05$ was used as the criterion of significance.

RESULTS

Rapid i.v. administration of FUra generates $FUraH_2$ concentrations of between 0.1 and 1 μg/ml within 5 min (Fig. 1). FUra concentrations declined rapidly within the first 15 min after administration whereas those of $FUraH_2$ steadily increased during the first 30 min. Concentrations of $FUraH_2$ exceeded those of FUra approximately 1 h after drug administration as a consequence of a lower clearance of $FUraH_2$. At 480 min after starting drug treatment, $FUraH_2$ was still detectable, whereas FUra concentration at t=240 min was below the limit of detection.

Differences between FUra and $FUraH_2$ concentrations of the four treatment groups generally became evident at t=60 min, both at day 1 and day 8. In CMF-treated rats protracted $FUraH_2$ levels were demonstrated at both day 1 and day 8. In CF-treated rats this was only found at day 8. In comparing plasma concentrations of both compounds over all time points, those of CMF-treated rats were significantly higher than those of CF-treated rats at day 1 (Friedman two-way analysis of variance). At day 8, concentrations of FUra and $FUraH_2$ in CF- and CMF-treated rats were significantly higher than those in F- and MF-treated rats. In comparing concentrations of FUra and $FUraH_2$ between day 1 and day 8 within treatment

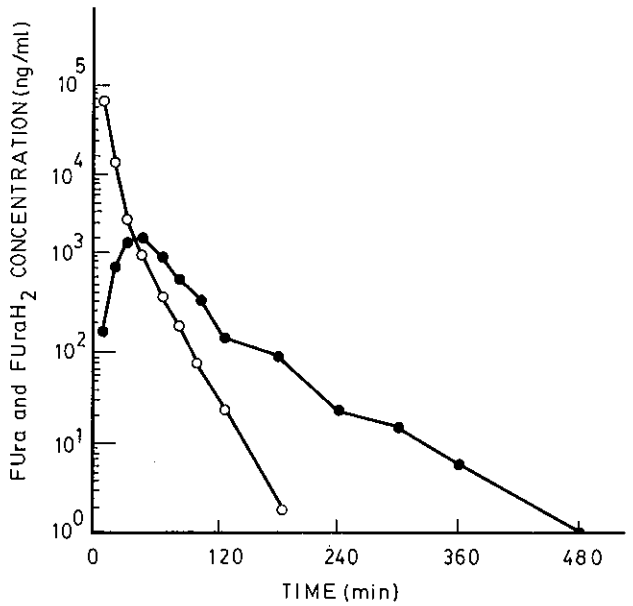

Fig. 1. Representative examples of $FUraH_2$ (●) and FUra (○) plasma concentration-time curves in a rat treated with CY (7 mg/kg×14 days, p.o.), MTX (2 mg/kg, days 1 and 8, i.v.) and FUra (20 mg/kg, days 1 and 8, i.v.). Compounds of interest were monitored at day 8 of the CMF regimen.

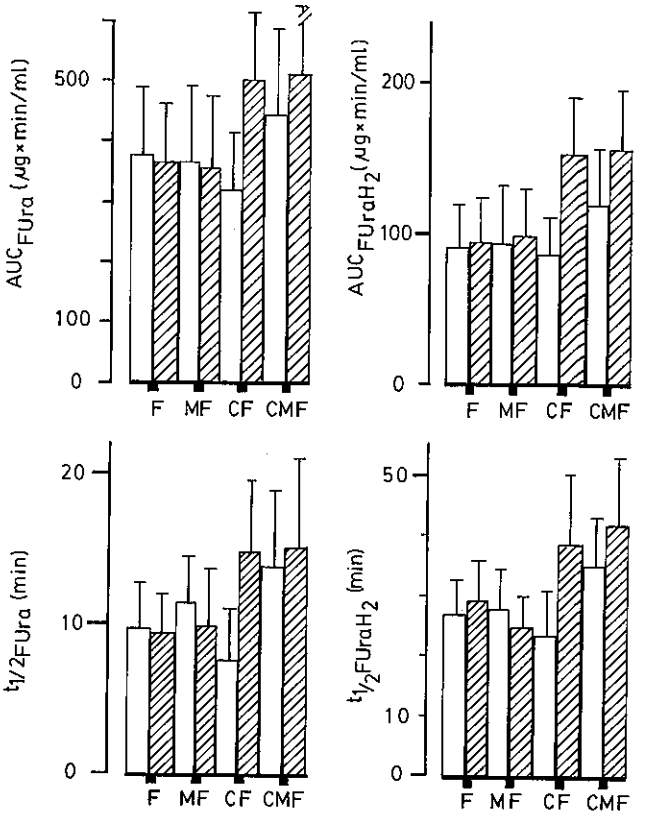

Fig. 2. AUC and $t_{1/2}$ of FUra and $FUraH_2$ in F-, MF-, CF- and CMF-treated rats at the first day (□) and eighth day (▨) of treatment according to the CMF regimen in the rat (24).

groups, the only significant change was found in the CF-treatment group: concentrations of both compounds were higher at day 8.

Pharmacokinetic data determined by non-compartmental analysis are shown in Figure 2 for FUra and $FUraH_2$ at day 1 and day 8 of the CMF regimen.

Pharmacokinetic data of the first day The mean AUC of FUra was highest in CMF-treated rats, which was related to a prolonged mean $t_{1/2,z}$ of the antimetabolite. Furthermore, AUC and $t_{1/2,z}$ of $FUraH_2$ were significantly altered in CMF-treated rats as compared to other treatment-groups. In the CF-treatment group increased CL was noted in comparison to single agent FUra. However, the difference failed to reach the level of significance. $FUraH_2$ levels of F- and CF-treated rats were comparable. In comparing the data of the four treatment-groups, significant differences could be demonstrated in CL_{TB} and $t_{1/2,z}$ of FUra between CF- and CMF-treated rats. The AUC and $t_{1/2,z}$ of $FUraH_2$ of CMF-treated rats were significantly altered in comparison to those of all other treatment groups. Two group comparisons demonstrated an influence of concomitant MTX on the $t_{1/2,z}$ of FUra and $FUraH_2$ (F+CF vs. MF+CMF); in contrast, the difference after a one step exchange in the previous combination (F+MF vs. CF+CMF) was not significant. Using two group comparisons, no significant influence of CY (CF-group) or MTX (MF-group) on $FUraH_2$ behavior could be discriminated.

Pharmacokinetic data at the eighth day In both FUra and $FUraH_2$, AUC and $t_{1/2,z}$ in the CMF-and CF-groups were significantly different from those in the F- and MF-treatment groups. A significant influence of CY on FUra and $FUraH_2$ behavior could be distinguished (F+MF vs. CF+CMF). No significant differences among the maximum concentration (C_{max}) of $FUraH_2$ of different treatments were demonstrated.

Pharmacokinetic data of day 1 vs. day 8 (intra-group comparison) AUC and $t_{1/2,z}$ of both FUra and $FUraH_2$ were significantly increased in CF-treated rats at day 8. AUC and $t_{1/2,z}$ of $FUraH_2$ in CMF-treated rats were also increased; however, the differences failed to reach significance. AUC and $t_{1/2,z}$ of FUra and $FUraH_2$ in F- and MF-treated rats at day 1 and day 8 were comparable. Remarkably, no significant differences in C_{max} of $FUraH_2$ could be demonstrated between day 1 and day 8 in any treatment group.

DISCUSSION

The CMF regimen is among the most frequently applied chemotherapeutic approaches in clinical oncology.[5] Pharmacokinetics of single agent CY, MTX and FUra have been described extensively: however, only a few reports deal with pharmacokinetic data of all the drugs included, and detailed data on interactions between the agents can only originate from animal studies.[14-17]

Data on *in vivo* kinetics of $FUraH_2$, the first product of FUra catabolism, are limited owing to the instability of the catabolite and difficulties of its determination.[19] In addition to data on the behavior of unchanged drugs and all possible interactions at the first day of the CMF regimen,[15-17] we report here on the consequences of concomitant CY and/or MTX for the behavior of FUra and its quantitatively most important metabolite $FUraH_2$, at the first and the eighth day of the CMF regimen.

Influences of CY and MTX on the behavior of FUra at the first day of the CMF regimen found in the present study were comparable to those reported earlier.[16] When the drugs were administered in combination (CMF), CL_{TB} was decreased and $t_{1/2,z}$ was prolonged. This could not have been predicted from the data obtained in CF- and MF-treated rats and it can be stated that changes of FUra behavior on the first day in the CMF-treatment group are associated with a specific interaction profile for the triple combination. Decreased CL_{TB} and prolonged $t_{1/2,z}$ of FUra have been demonstrated to be associated with increased AUC and prolonged $t_{1/2,z}$ of $FUraH_2$ while C_{max} of the catabolite remained unchanged.

This is suggestive of a decrease of $FUraH_2$ breakdown rate at day 1 in CMF-treated rats only, resulting in decreased CL_{TB} of FUra and prolonged $t_{1/2,z}$ of both FUra and $FUraH_2$. Decrease of $FUraH_2$ clearance might be caused by inhibition of further catabolic breakdown or decrease of renal clearance of catabolites.[13] Decreased urinary elimination of FUra catabolites such as FUPA and FBAL owing to the presence of MTX[13] and urotoxic CY metabolites, the latter being rapidly formed after CY administration,[20] is amongst the possible explanations of elevated FUra and $FUraH_2$ levels in the CMF regimen. Interestingly, C_{max} of $FUraH_2$ remained comparable over all treated groups. The background of this phenomenon needs to be clarified, but it seems unlikely that altered enzymatic degradation of FUra and $FUraH_2$ is already involved at the first day of the CMF regimen. This, however, is difficult to measure since $FUraH_2$ in urine is subject to rapid chemical degradation into FUPA and FBAL. It might well be that FUPA/FBAL urinary data do not precisely reflect hepatic FUra catabolism. The main feature of the present findings is that systemic $FUraH_2$ levels are sustained upon repeated CY/MTX administrations. This might in part explain unexpected toxicity encountered in the CMF regimen. Data on creatinine clearance of the treatment groups were not suggestive of an important role of altered renal functions in the changes of systemic FUra and $FUraH_2$ behavior presently encountered. The AUC of FUra is increased 20–25%, and therefore, it can be stated that interactions at the pharmacokinetic level contribute to the efficacy of CY+MTX+FUra (i.e., the CMF regimen).

At the eighth day of the CMF regimen, CY has been administered eight times and the antimetabolites twice. Effects of induction or inhibition of enzymatic systems, especially by CY, might then become evident. The data of the present study demonstrate that changes of FUra and $FUraH_2$ owing to repeated concomitant CY occur, but are not dramatic (F+MF vs. CF+CMF, eighth day). The most profound change was demonstrated in CF-treated rats: AUC values of FUra and $FUraH_2$ were the lowest at the first day while significant increases were found at the eighth day (CF, 1st day vs. 8th day). FUra and $FUraH_2$ levels in CF rats then became comparable to those in CMF-treated rats. Increase of AUC was associated with prolonged $t_{1/2,z}$ of both FUra and $FUraH_2$. The mechanism of the changes of CL_{TB} of FUra and $FUraH_2$ at the eighth day also need to be clarified, but are most likely related to previous, repeated CY administration. Differences between FUra and $FUraH_2$ in the CMF-treatment group at day 1 and day 8 were small and failed to reach the level of significance. Furthermore, it should be stressed that the same interactions as noted at the first day in the CMF-treatment group may still contribute to elevated levels of FUra and $FUraH_2$ at the eighth day in the regimen. In any case, the elevated levels of FUra at day 8 contribute to the efficacy of CMF and CF as they represent a masked dose increase.

With respect to FUra activity, increased activity of the fluoropyrimidine in the CMF-treatment group might be expected since systemic exposure to FUra (AUC), i.e., the dose, is increased even at the first day of the CMF regimen, probably owing to an altered renal excretion of FUra catabolites. It should be stressed, however, that 1) formation of active metabolites of FUra in tumor cells is required for increased efficacy 2) toxicity is also AUC-related and 3) *in vivo* behavior of MTX and CY can also be changed in CMF-treated rats when compared to other treatment possibilities.[15,17]

In our previous studies we were able to demonstrate that CL_{TB} of MTX increased more than 43%[15] whereas the AUC of CY increased 50%[17] upon stepwise combinations of MTX with CY and FUra and of CY with MTX and FUra, respectively. When AUC of MTX is decreased, lower efficacy might be expected because of the activity of the drug itself. For interpretation of changes of CY behavior, however, monitoring of metabolic activation of CY is mandatory.

Our studies have implications for comparative studies in breast cancer patients receiving CY, MTX and FUra alone, or in different combinations.[8, 10] Taking into account the special interactions at the pharmacokinetic level, masked beneficial effects can be missed when one or two drugs are omitted from the CMF regimen. Removal of CY, for instance, as in MF treatment,[10] might have more impact on treatment efficacy than expected since not only is the influence of CY and its metabolites on extratumoral behavior of MTX and FUra, and thus on effective dose of the antimetabolites, lacking,[15–17] but also the influence of CY and metabolites on intratumoral behavior of MTX and FUra[21] and the additional immunogenic activity of CY are lost.[22, 23] Therefore, comparative studies on the treatment of breast cancer which include different combinations of CY, MTX and FUra should be interpreted with great care.[9] Masked differences in drug disposition might lead to lowering or even lack of efficacy, including toxicity, especially after administration of MTX+FUra.[10, 24] Moreover, limited drug monitoring should be included in studies with different combinations in order to reveal differences between drug disposition among the regimens applied.[9]

Interactions at the biochemical level between CY, MTX and FUra with emphasis on MTX and FUra have been reviewed recently.[21] The general assumption is that possible synergism between CY, MTX and FUra is determined by intracellular interactions and intrinsic sensitivity of tumor cells exposed to the respective drugs. In this study, we have established the relationship between FUra systemic exposure and concomitant CY and MTX. The present data are suggestive of an alternative pathway of synergism between drugs used in the CMF regimen, including a masked change of the dose owing to interactions at the pharmacokinetic level.

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REFERENCES

1) Cooper, R. Combination chemotherapy in hormone resistant breast CA. *Proc. Am. Assoc. Cancer Res. Am. Soc. Clin. Onc.*, **10**, 15 (1969).
2) Davis, H. L., Jr., Ramirez, G., Ellerby, R. A. and Ansfield, F. J. Five-drug therapy in advanced breast cancer: factors influencing toxicity and response. *Cancer*, **34**, 239–245 (1974).
3) Canellos, G. P., Devita, V. T., Gold, G. L., Chabner, B. A., Schein, P. S. and Young, R. C. Combination chemotherapy for advanced breast cancer. Patterns of response and effect on survival. *Ann. Intern. Med.*, **84**, 389–392 (1976).
4) Canellos, G. P., Pocock, S. J., Taylor, S. G., III, Sears, M. E., Klaasen, D. J. and Band, P. R. Combination chemotherapy for metastatic breast carcinoma. Prospective comparison of multiple drug therapy with L-phenylalanine mustard. *Cancer*, **38**, 1882–1886 (1976).
5) Bonadonna, G. and Valagussa, P. The contribution of medicine to the primary treatment of breast cancer. *Cancer Res.*, **48**, 2314–2324 (1988).
6) Bonadonna, G. and Valagussa, P. Dose-response effect of adjuvant chemotherapy in breast cancer. *N. Engl. J. Med.*, **304**, 10–15 (1981).
7) Bonadonna, G. Conceptual and practical advances in the management of breast cancer. *J. Clin. Oncol.*, **7**, 1380–1397 (1989).
8) National Institutes of Health. Adjuvant chemotherapy for breast cancer. *In* "Consensus Development Conference Statement," Vol. 5, No. 12 (1985). Bethesda, Maryland.
9) Peto, R. and Early Breast Cancer Trialists Collaborative Group. Effects of adjuvant tamoxifen and cytotoxic therapy on mortality in early breast cancer. An overview of 61 randomized trials among 28,896 women. *N. Engl. J. Med.*, **319**, 1682–1692 (1988).
10) Fisher, B., Redmond, C. and Dimitrov, N. W. A randomized clinical trial evaluating sequential methotrexate and fluorouracil in the treatment of patients with node-negative breast cancer who have estrogen-receptor negative tumors. *N. Engl. J. Med.*, **320**, 473–478 (1989).
11) Pinedo, H. M. and Peters, G. F. J. Fluorouracil: biochemistry and pharmacology. *J. Clin. Oncol.*, **6**, 1653–1664 (1988).
12) Diasio, R. B. and Harris, B. E. Clinical pharmacology of 5-fluorouracil. *Clin. Pharmacokinet.*, **16**, 215–237 (1989).
13) De Bruijn, E. A., Van Oosterom, A. T. and Tjaden, U. R. Site-specific delivery of 5-fluorouracil with 5-deoxy-5-fluorouridine. *Reg. Cancer Treat.*, **2**, 61–76 (1989).
14) Slee, P. H. Th. J., De Bruijn, E. A., Driessen, O. M. J., Hermans, J. and Van Oosterom, A. T. Pharmacokinetics of the cytostatic drugs used in the CMF-regimen. *Anticancer Res.*, **3**, 269–272 (1983).
15) De Bruijn, E. A., Driessen, O., Leeflang, P. A., Van Den Bosch, N., Van Strijen, E., Slee, P. H. Th. J. and Hermans, J. Pharmacokinetic interactions of cyclophosphamide and 5-fluorouracil with methotrexate in an animal model. *Cancer Treat. Rep.*, **70**, 1159–1165 (1986).
16) De Bruijn, E. A., Driessen, O., Leeflang, P., Van Strijn, E., Van Den Bosch, N. and Hermans, J. Interactions of methotrexate and cyclophosphamide with the pharmacokinetics of 5-fluorouracil in an animal model. *Cancer*

Treat. Rep., **71**, 1267–1269 (1987).
17) De Bruijn, E. A., Geng, Y., Hermans, J. and Driessen, O. The CMF-regimen. Modulation of cyclophosphamide uptake and clearance by methotrexate and fluorouracil. *Int. J. Cancer*, **45**, 935–939 (1990).
18) De Bruijn, E. A., Driessen, O., Van Den Bosch, N., Van Strijen, E., Slee, P. H. Th. J., Van Oosterom, A. T. and Tjaden, U. R. A gas chromatographic assay for the determination of 5,6-dihydrofluorouracil and 5-fluorouracil in human plasma. *J. Chromatogr.*, **278**, 283–289 (1983).
19) De Bruijn, E. A., Remeyer, L., Tjaden, U. R., Erkelens, C., De Brauw, L. M. and Van De Velde, C. J. H. Non-linear pharmacokinetics of 5-fluorouracil as described by *in vivo* behaviour of 5,6-dihydro-5-fluorouracil. *Biochem. Pharmacol.*, **35**, 2461–2465 (1986).
20) Natarajan, A. T., De Bruijn, E. A., Leeflang, P., Slee, P. H. Th. J., Mohn, G. R. and Driessen, O. Induction of chromosomal alterations as an assay for cytostatic drugs activity in plasma. *Mutat. Res.*, **121**, 39–45 (1983).
21) Damon, L. E., Cadman, E. and Benz, C. Enhancement of 5-fluorouracil antitumor effects by the prior administration of methotrexate. *Pharmacol. Ther.*, **43**, 155–185 (1989).
22) Kawabata, T. T. and White, K. L., Jr. Enhancement of *in vivo* and *in vitro* murine immune responses by the cyclophosphamide metabolite acrolein. *Cancer Res.*, **48**, 41–45 (1988).
23) Berd, D. and Mastranglo, M. J. Effect of low dose cyclophosphamide on the immune system of cancer patients: depletion of CD4+, 2H4+ suppressor-inducer T-cells. *Cancer Res.*, **48**, 1671–1675 (1988).
24) De Bruijn, E. A., Driessen, O. M. J. and Hermans, J. The CMF-regimen. Toxicity patterns following stepwise combinations of cyclophosphamide, methotrexate and fluorouracil. *Int. J. Cancer*, **48**, 67–72 (1991).