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Stereospecific pharmacokinetics of *rac*-5-methyltetrahydrofolic acid in patients with advanced colorectal cancer

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1 The pharmacokinetics and toxicity of racemic 5-methyltetrahydrofolic (*rac*-5-MTHF) acid after i.v. infusion were investigated in 18 patients with advanced colorectal cancer. Doses of 100–600 mg *rac*-5-MTHF/m^2 were administered over 2 h together with a bolus of 500 mg/m^2 5-fluorouracil (5-FU) as a midpoint injection.

2 The pharmacokinetics of both diastereoisomers were linear in the range from 100–600 mg 5-MTHF/m^2. Independent of the administered dose, the maximal plasma concentration of [R]-5-MTHF was nearly twice that of [S]-5-MTHF. The elimination of [S]-5-MTHF from plasma was considerably faster than that of the [R]-isomer (elimination half-life: 3.1 ± 1.0 h *vs* 8.3 ± 3.2 h). No metabolites were detected in plasma and in urine samples.

3 The plasma protein binding was stereoselective ([R]-5-MTHF bound: 88.2 ± 2.7%; [S]-5-MTHF bound: 59.9 ± 6.8%; $P < 0.001$), causing a significantly higher renal clearance for [S]-5-MTHF when compared with the [R]-isomer (37.5 ± 23.7 ml min^{-1} *vs* 12.7 ± 11.2 ml min^{-1}, $P < 0.001$). There was no dose dependence, but gender influenced renal clearance (CL_{ren} [R]-5-MTHF: male *vs* female: 20.5 ± 14.5 ml min^{-1} *vs* 7.8 ± 4.7 ml min^{-1}, $P = 0.03$; CL_{ren} [S]-5-MTHF: male *vs* female: 57.2 ± 21.7 ml min^{-1} *vs* 25.7 ± 16.2 ml min^{-1}, $P = 0.006$).

4 Toxic side effects of the combination 5-FU/5-MTHF were rare and generally mild, and included stomatitis, nausea/emesis, diarrhoea, anaemia, leukopenia, and thrombocytopenia.

5 In combination with 500 mg 5-FU/m^2 a single dose of 600 mg *rac*-5-MTHF/m^2 can safely be administered to patients with colorectal cancer. A similar therapeutic benefit of 5-MTHF to folinic acid in the biochemical modulation of 5-FU is supported by the comparison of *in vitro* and *in vivo* data.

Keywords pharmacokinetics colorectal cancer stereospecificity 5-methyltetrahydrofolic acid

Introduction

The biochemical modulation of 5-FU by folinic acid is a standard regimen in the palliative treatment of colorectal cancer. The pharmacokinetics of folinic acid have demonstrated the nearly quantitative metabolism of folinic acid to 5-MTHF after oral and intravenous administration of 25 mg folinic acid/m^2 [1]. Even in high-dose regimens with a 2 h infusion of 600 mg folinic acid/m^2 metabolism amounts to 40% of the administered dose [2, 3]. Preclinical data emphasise an active role of 5-MTHF in the modulation of 5-FU [4–6]. In lymphoid leukaemia cells a cytocidal effect of 5-MTHF alone was observed at a concentration of 0.5 mM, which did not impair the viability of normal human lymphocytes [7]. Intracellular expansion of folate pools by 5-MTHF has been deduced from the use of 5-MTHF as a rescue

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agent after high-dose therapy with methotrexate [8–11]. Beyond that, intravenous administration of folinic acid as a rescue agent resulted in prolonged persistence of the metabolite 5-MTHF in cerebrospinal fluid, preventing the undesired side effects of methotrexate on the central nervous system after intrathecal administration [12, 13].

In the treatment of colorectal cancer, the role of 5-MTHF as a biochemical modulator of 5-FU has been evaluated in only a few studies with contradictory results [14–16]. Hitherto, no pharmacokinetic study focusing on the diastereoisomers [R]-5-MTHF and [S]-5-MTHF has been performed, although the analytical separation of both isomers by chiral h.p.l.c. has been described [17]. Highly stereoselective pharmacokinetics of [R]-folinic acid and [S]-folinic acid were observed after oral and intravenous administration of *rac*-folinic acid, suggesting a stereospecific behaviour of *rac*-5-MTHF.

In the present study, patients with advanced colorectal cancer received 500 mg 5-FU/m^2 together with escalating doses of *rac*-5-MTHF by i.v. infusion. The aim was to investigate the stereoselectivity of *rac*-5-MTHF disposition including plasma kinetics, plasma protein binding, and urinary excretion of [R]- and [S]-5-MTHF, as well as to document toxicity under concomitant administration of 5-FU and escalating doses of 5-MTHF.

Methods

Patient eligibility

The study protocol was approved by the institution's ethics committee and patients with histologically confirmed advanced colorectal cancer (Table 1) entered the study after informed consent was obtained. Patients had fully recovered from the toxic effects of prior systemic therapy. Prior radiotherapy was completed at least 4 weeks before study entry. Prior to study entry each patient underwent complete laboratory investigation. Only patients with normal renal and hepatic function (serum creatinine < 1.3 mg% and BUN 6–25 mg%, serum bilirubin < 1 mg%, ALT < 23 u l^{-1}, AST < 18 u l^{-1}, and gamma-GT 6–28 u l^{-1}, respectively) were included.

Treatment protocol

Eighteen patients were treated with a single dose of 500 mg 5-FU/m^2 (Fluorouracil®, Cehasol ABIC, Netanya, Israel) and with 100–600 mg *rac*-5-MTHF/m^2 (SAPEC Inc., Lugano, Switzerland; kindly supplied by Schoeller Pharma, Austria). The dose of *rac*-5-MTHF was escalated in steps of 100 mg/m^2 corresponding to a cautious version of the modified Fibonacci dose escalation schema [18]. *Rac*-5-MTHF was infused to three patients per dose level intravenously over 2 h with a midpoint bolus injection of 5-FU. Considering the lack of pharmacokinetic data and the limited clinical experience with 5-FU/MTHF, the initial dose of *rac*-5-MTHF was 100 mg/m^2. The observation of a WHO grade 3 toxicity of any kind in more than one patient at a given dose level was defined to terminate further dose escalation, as was the single occurrence of a WHO grade 4 toxicity. *Rac*-5-MTHF 600 mg/m^2 was defined as the upper dose limit of this study analogous to the treatment with the combination of 5-FU/*rac*-folinic acid. No antiemetics were given prophylactically. Patients were accepted in the study without randomisation, but were matched by age and sex.

Table 1 Patient characteristics

Number	18
Age (median of years (range))	66 (53–78)
Sex	
Female	10
Male	8
Primary tumour	
Colon cancer	11
Rectal cancer	7
Site of metastases	
Liver	11
Liver and lung	1
Liver and intestine	1
Liver and lymph nodes	1
Liver, lung and local recurrence	1
Lung	1
Local recurrence	1
Carcinosis peritonei	1
Previous therapy	
Surgical therapy	17
Radiotherapy	8
Chemotherapy	13
5-Fluorouracil/folinic acid	12*
5-Fluorouracil/Interferon α	1

*Two patients received second-line therapy with mitomycin C, and one patient with *cis*-platinum.

Analytical methods

Rac-5-MTHF (purity > 98%) as the chromatographic standard was kindly supplied by SAPEC Inc. Prior to the study the composition of *rac*-5-MTHF vials was evaluated, and the equal proportion of both isomers (50% [R]-5-MTHF and 50% [S]-5-MTHF) was confirmed by chiral h.p.l.c. Water for chromatographic purposes was purchased from Rathburn (Walkerburn, Scotland). All other substances were purchased from Merck (Darmstadt, Germany) and were of analytical grade.

Blood samples were collected prior to treatment, every 0.5 h during the 2 h infusion of *rac*-5-MTHF and 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 22 h after infusion completion. Blood samples were collected in EDTA-tubes, centrifuged and stabilised immediately (adding 50 µl of 5% ascorbic acid in water (w/v) to 1 ml plasma). The plasma was analysed within 1 h by h.p.l.c.

In order to determine the plasma protein binding 0.2 ml of fresh plasma (without ascorbic acid) was centrifuged immediately after collection through Ultrafree-MC filter units with a nominal cut-off of Mr 10 000 (Millipore Corporation, Molsheim, France)

and centrifuged for 15 min to separate free drug from protein bound drug. Except in the group with the lowest dose of *rac*-5-MTHF (100 mg/m^2), the plasma protein binding of the diastereoisomers was determined in all patients at the end of the infusion of *rac*-5-MTHF, and 1 h and 4 h later, respectively.

Urine was collected over 24 h (three to five samples) in containers with 1 ml of 5% ascorbic acid in water (w/v). An aliquot was then stored at −70° C until analysis, generally 2 days after collection.

Sample clean-up

Aliquots (10–20 µl) of the centrifuged plasma samples or ultrafiltrates were injected directly onto the h.p.l.c. without further pretreatment of the samples.

Centrifuged urine (0.2 ml) was diluted with 0.8 ml distilled water and applied to a strong anionic exchange sorbent (Bond Elut SAX, Analytichem Int., Harbor City, USA; 100 mg sorbent were conditioned with 1 ml methanol and 1 ml water). The column was then washed with 1 ml water, 1 ml methanol and the folates were eluted with 1 ml 1 M tri-sodium citrate buffer, pH = 7.5, containing 0.25% ascorbic acid. Aliquots (10–20 µl) of the eluate were injected directly onto the h.p.l.c.

Chromatography

[R]-5-MTHF and [S]-5-MTHF were separated on Resolvosil BSA (150 × 4 mm, 7 µm particles obtained from Macherey-Nagel, Dueren, Germany) as the stationary phase. Sodium/potassium phosphate buffer 50 mM, pH = 7.5, was used for the isocratic elution of the compounds from plasma samples with a flow rate of 1 ml min^{-1}. Typical retention times were 6.4 min for [R]-5-MTHF and 11.6 min for [S]-5-MTHF.

Urine samples were analysed by a gradient from 5 mM to 50 mM ion strength between 1 and 12 min with a flow rate of 1 ml min^{-1}. Typical retention times were 7.2 min for [R]-5-MTHF and 12.7 min for [S]-5-MTHF.

Quantitation of the folates was by external standard calibration measuring the absorbance of [R]-5-MTHF and [S]-5-MTHF at 287 nm.

Quality control

Four concentrations of *rac*-5-MTHF were prepared in plasma (2, 10, 100 and 1000 µg ml^{-1}) and were assayed as described in the anlaytical section. The linearity of the standard curve was excellent (coefficient of correlation r = 0.999 for both isomers). The lower limit of quantitation was 1 µg ml^{-1} plasma for each isomer. The mean recovery of [S]-5-MTHF was 99.2% (mean coefficient of variation in the investigated range: 2.9%) and 98.8% for [R]-5-MTHF (mean coefficient of variation in the investigated range: 1.6%) when compared with aqueous preparations of the drug. The mean recoveries at the lower limit of quantitation were 98.8% for [R]-5-MTHF and 97.5% for [S]-5-MTHF.

Plasma and urine samples stored at −70° C were thawed and re-evaluated. When stabilised with ascorbic acid, [R]-5-MTHF and [S]-5-MTHF were found to be stable up until 2 months after collection of the samples.

Analysis of data

Data points were fitted by non-linear least squares regression without weighting of data using the program PKCALC to obtain the rate constants [19]. AUC values were calculated by exponential equation. Plasma clearance (CL_p) was calculated from Dose/AUC, with Dose = administered dose of [R]-5-MTHF or [S]-5-MTHF. Renal clearance (CL_{ren}) was calculated from *Ae*/AUC with *Ae*0,24 = amount excreted into urine within 24 h. The fraction of plasma protein bound drug was calculated by conventional methods.

For statistical evaluations, pharmacokinetic parameters were compared using the Wilcoxon test and the Kruskal-Wallis test (nonparametric comparisons of data sets from single dose groups), and the one way analysis of variance (evaluation of dose independent parameters in 18 subjects).

Results

Pharmacokinetics

After administration of *rac*-5-MTHF, the maximal plasma concentration of [R]-5-MTHF was nearly twice that of [S]-5-MTHF, irrespective of the administered dose (Table 2). The elimination of [S]-5-MTHF was considerably faster than the elimination of the [R]-isomer: 24 h after the start of the 2 h infusion of *rac*-5-MTHF, no [S]-5-MTHF was detected in plasma, whereas [R]-5-MTHF was present in all specimens. In contrast to the elimination half-lives, the short distribution phase after the end of the infusion was similar in length for both compounds (Figure 1). The distribution half-life and the elimination half-life of both isomers were independent of the

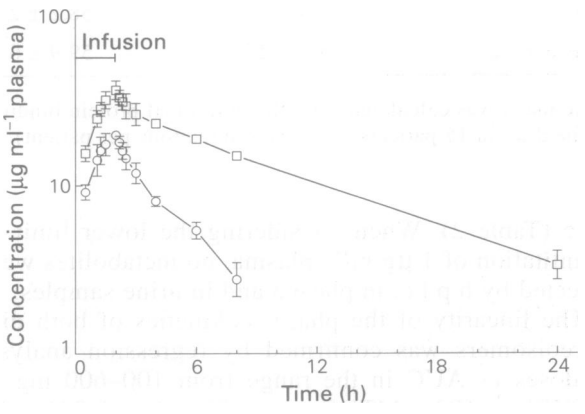

Figure 1 Concentration-time profile of [R]-5-MTHF (□) and [S]-5-MTHF (○) after intravenous administration of 500 mg *rac*-5-MTHF/m^2 as a 2 h infusion (mean plasma concentrations ± s.d., n = 3).

Table 2 Mean (± s.d.) pharmacokinetic parameters describing the fate of the diastereoisomers of 5-MTHF after a 2 h i.v. infusion of 100–600 mg *rac*-5-MTHF/m^2 (three patients per dose level)

	[R]-5-MTHF				*[S]-5-MTHF*			
rac-*5-MTHF* *(mg/m^2)*	C_{max} *(μg ml^{-1})*	*AUC (μg ml^{-1} h)*	$t_{1/2,\lambda_1}$ *(h)*	$t_{1/2,z}$ *(h)*	C_{max} *(μg ml^{-1})*	AUC *(μg ml^{-1} h)*	$t_{1/2,\lambda_1}$ *(h)*	$t_{1/2,z}$ *(h)*
100	6.3 ± 0.42	78.5 ± 22.6	0.69 ± 0.20	12.1 ± 5.4	4.3 ± 0.57	22.4 ± 2.1	0.39 ± 0.33	3.9 ± 0.8
200	16.5 ± 5.89	157 ± 113	0.36 ± 0.07	7.3 ± 3.9	10.7 ± 4.3	49.9 ± 32.3	0.27 ± 0.02	3.4 ± 1.8
300	20.3 ± 1.88	237 ± 28.3	0.52 ± 0.49	8.8 ± 0.8	13.2 ± 1.3	69.1 ± 8.9	0.76 ± 0.49	3.7 ± 0.8
400	32.9 ± 1.80	281 ± 38.4	0.56 ± 0.19	7.0 ± 0.8	18.5 ± 1.5	70.6 ± 13.9	0.20 ± 0.13	2.2 ± 0.3
500	37.0 ± 4.54	339 ± 24.3	0.37 ± 0.14	7.2 ± 0.9	20.0 ± 1.2	84.4 ± 13.6	0.46 ± 0.38	2.7 ± 0.54
600	38.9 ± 10.6	420 ± 225	0.63 ± 0.63	7.6 ± 3.2	25.0 ± 7.1	117 ± 43.3	0.35 ± 0.24	2.9 ± 0.9
Mean ± s.d.	n.a.	n.a.	0.52 ± 0.32	8.3 ± 3.2	n.a.	n.a.	0.40 ± 0.32	3.1 ± 1.0

*The mean value was calculated from 18 individual model fits.
n.a. = not applicable.

Table 3 Plasma clearance, renal clearance, and cumulative urinary excretion after a 2 h infusion of 100–600 mg *rac*-5-MTHF/m^2 (mean ± s.d. values are reported for three patients per dose level)

	[R]-5-MTHF			*[S]-5-MTHF*		
rac-*5-MTHF* *(mg/m^2)*	CL_p *(ml min^{-1})*	CL_{ren} *(ml min^{-1})*	Ae*24h* *(%)*	CL_p *(ml min^{-1})*	CL_{ren} *(ml min^{-1})*	Ae*24h* *(%)*
100	17.8 ± 3.7	4.7 ± 2.2	25.0 ± 9.5	60.5 ± 9.4	21.0 ± 18.7	34.0 ± 32.1
200	24.7 ± 15.2	13.2 ± 12.0	48.3 ± 16.6	71.4 ± 35.6	35.9 ± 20.3	48.3 ± 5.5
300	21.0 ± 4.1	9.3 ± 3.5	44.5 ± 5.0	72.3 ± 14.9	30.3 ± 17.9	40.5 ± 13.4
400	20.5 ± 3.5	11.5 ± 7.0	53.7 ± 28.3	83.2 ± 20.6	35.7 ± 22.4	41.0 ± 21.7
500	23.7 ± 1.7	14.2 ± 2.3	59.7 ± 7.2	95.4 ± 2.8	47.2 ± 10.8	49.7 ± 12.6
600	33.0 ± 30.7	27.2 ± 28.0	55.5 ± 19.1	93.5 ± 59.5	59.4 ± 56.0	45.5 ± 23.3
Mean ± s.d.	23.5 ± 13.0	12.7 ± 11.2	47.5 ± 18.4	79.3 ± 28.7	37.5 ± 23.7	43.2 ± 17.5

Table 4 Plasma protein binding of [R]-5-MTHF and [S]-5-MTHF after administration of *rac*-5-MTHF (mean ± s.d. values of three patients per dose level, calculated from three determinations per patient, 2, 3 and 6 h after the start of the infusion, respectively)

rac-*5-MTHF* *(mg/m^2)*	*[R]-5-MTHF* *% bound*	*[S]-5-MTHF* *% bound*
200	91.8 ± 3.1	65.6 ± 14.8
300	88.8 ± 1.1	56.0 ± 3.0
400	87.7 ± 0.8	59.3 ± 1.6
500	87.1 ± 1.6	60.3 ± 3.4
600	85.4 ± 1.6	58.2 ± 2.4
*Mean ± s.d.	88.2 ± 2.7	59.9 ± 6.8

*The mean was calculated from the individual protein binding of the drug in 15 patients (three determinations per patient).

dose (Table 2). When considering the lower limit of quantitation of 1 μg ml^{-1} plasma, no metabolites were detected by h.p.l.c. in plasma and in urine samples.

The linearity of the pharmacokinetics of both diastereoisomers was confirmed by regression analysis of doses *vs* AUC in the range from 100–600 mg 5-MTHF/m^2 ([R]-5-MTHF: $r = 0.996$, $P < 0.001$; [S]-5-MTHF: $r = 0.969$, $P = 0.001$). A linear relationship was also observed between the maximal plasma concentrations and the administered dose.

The total plasma clearance was independent of the administered dose with a mean value of 23.5 ± 13.0 ml min^{-1} for [R]-5-MTHF and 79.3 ± 28.7 ml min^{-1} for [S]-5-MTHF ($P < 0.0001$; 95% confidence interval of the difference: 40.7–70.9 ml min^{-1}; Table 3). The slope of the regression analysis of the maximal plasma concentration *vs* the total plasma clearance or the renal clearance did not differ significantly from zero.

The plasma protein binding of [R]-5-MTHF and [S]-5-MTHF was evaluated at the end of the infusion, then 1 and 4 h later. A higher affinity of the [R]-isomer was observed ([R]-5-MTHF bound: 88.2 ± 2.7% [S]-5-MTHF bound: 59.9 ± 6.8%; $P < 0.001$; 95% confidence interval of the difference: 24.4–32.2%). The protein binding hardly changed over this time period (differences between three consecutive determinations in 15 patients: [R]-5-MTHF: 0.4–6.9%; [S]-5-MTHF: 3.7–8.6%). The plasma protein binding was independent of the dose, and showed little interpatient variance (Table 4). The slight decrease of [R]-MTHF bound to plasma protein at higher doses of *rac*-MTHF did not reach statistical significance.

The renal clearance was significantly higher for [S]-5-MTHF when compared with the [R]-isomer (37.5 ± 23.7 ml min^{-1} *vs* 12.7 ± 11.2 ml min^{-1}, $P < 0.001$; 95% confidence interval of the difference: 12.2–37.4 ml min^{-1}). There was a noteworthy influence of drug stereochemistry and gender on the renal clearance, which was more than doubled in

male patients (renal clearance of [R]-5-MTHF: male *vs* female: 20.5 ± 14.5 ml min^{-1} *vs* 7.8 ± 4.7 ml min^{-1}, $P = 0.03$; 95% confidence interval of the difference: 2.2–23.2 ml min^{-1}; renal clearance of [S]-5-MTHF: male *vs* female: 57.2 ± 21.7 ml min^{-1} *vs* 25.7 ± 16.2 ml min^{-1}, $P = 0.006$; 95% confidence interval of the difference: 11.2–51.8 ml min^{-1}).

However, the difference in renal clearance of the [R]- and [S]-isomers disappeared when adjusted to the unbound plasma fraction of the drug. The mean renal clearance of the unbound isomers was approximately that of creatinine in normal subjects (CL_{ren} free [R]-MTHF: 140 ml min^{-1}; CL_{ren} free [S]-MTHF: 114 ml min^{-1}).

Independent of the administered dose, the most variable parameters were the renal clearance and the amount of drug excreted within 24 h (47.5 ± 18.4% of administered [R]-5-MTHF; 43.2 ± 17.5% of administered [S]-5-MTHF; see Table 3).

Toxicity

Toxicities were rare and included only one patient with stomatitis WHO grade 3 (i.e. ulcerations, fluid nutrition necessary) at the highest dose level of *rac*-5-MTHF. One patient suffered from a pulmonary infection (WHO grade 3, i.e. severe infection, parenteral antibiotics required) 2 weeks after therapy, which was probably not related to 5-FU/*rac*-5-MTHF, since no leukocyte depression was observed within this period. Nausea/vomiting occurred in two patients (WHO grade 2, i.e. transient nausea/vomitus, no antiemetics necessary). Other toxicities which were of no clinical relevance concerned diarrhoea in one patient (WHO grade 1, i.e. transient, < 2 days), anaemia in one patient (WHO grade 1, i.e. haemoglobin between 9.5 and 10.9 mg%), leukopenia in one patient (WHO grade 1, i.e. leukocytes between 3000 ml^{-1} and 3900 ml^{-1}), and thrombocytopenia in one patient (WHO grade 1, i.e platelets between 75 000 ml^{-1} and 99 000 ml^{-1}). Due to the low incidence of side effects, a relationship between toxicity and dose could not be established.

Discussion

Although the stereospecific metabolism of 5-MTHF is not fully understood, there is some evidence for a notable difference between the [R]- and the [S]-isomer. Metabolism under physiological conditions involves only the naturally occurring compound [S]-5-MTHF, which is also the only circulating active metabolite after administration of *rac*-folinic acid. With the exception of a high serum albumin binding of [R]-5-MTHF, no relevant interaction of this diastereoisomer has been described in biological systems.

The therapeutic use of the combination 5-FU and tetrahydrofolate aims at thymidylate synthase inhibition via a ternary complex with fluorodeoxyuridinemonophosphate (FdUMP) and $N^{5,10}$-methylenetetrahydrofolate. Beyond the known implication of $N^{5,10}$-methylenetrahydroflate, the binding of FdUMP to thymidylate synthase was stable in the presence of tetrahydrofolic acid, suggesting several folate compounds to promote the thymineless death of tumour cells. Lower activity was observed for dihydrofolic acid and 5-MTHF, whereas folinic acid did not facilitate the association [20].

The main route of metabolism of 5-MTHF in mammalian cells leads to tetrahydrofolic acid, which is an excellent substrate for folylpolyglutamate synthetase. This enzyme elongates the glutamate side chain of tetrahydrofolates and prevents folate efflux from cells. This is in agreement with the lack of circulating metabolites after administration of *rac*-5-MTHF. The stereospecific resolution offered by Resolvosil columns, however, implies a detection limit of 1 μg ml^{-1}, which is obviously too high for the detection of folates circulating at low concentrations in blood. In this context, the increased uptake of 5-MTHF into tumour cells together with the subsequent metabolism by folylpolyglutamate synthetase suggests that 5-MTHF behaves like a prodrug of tetrahydrofolic acid.

The concentration-time profile of 5-MTHF in plasma shares prominent features with folinic acid: a faster elimination of the [S]-isomer from blood, a higher renal clearance of the [S]-form, and a high stereoselective plasma protein binding of the [R]-isomer [21]. In the case of [S]-folinic acid, this phenomenon is attributable almost entirely to metabolism, whereas [R]-folinic acid is metabolically inert. Comparing [R]-5-MTHF with [S]-5-MTHF, the elimination from blood seems to be due primarily to renal clearance, which is determined by the different plasma protein binding of both isomers.

Differences in the tetrahydrofolates include a monoexponential decay of [R]-folinic acid after administration of *rac*-folinic acid with an elimination half-life ranging from 8.3 to 9.9 h [3, 22], and the biexponential decay of [S]-5-MTHF with a short distribution phase up to 30 min after infusion completion, and a mean elimination half-life of 3.1 h. The elimination half-life of metabolically generated [S]-5-MTHF after administration of folinic acid is considerably longer (6–10 h) when compared with intravenously administered *rac*-5-MTHF, indicating a depot function with slow release for the liver as the metabolising tissue after intravenous administration [2, 3, 22]. Due to extensive metabolism of [S]-folinic acid, the plasma clearance of [S]-folinic acid was higher when compared with that of [S]-5-MTHF. The differences in the renal clearance of [S]-folinic acid and [S]-5-MTHF can be explained by different plasma protein binding (54.9 ± 22.0 ml min^{-1} [3] and 37.5 ± 23.7 ml min^{-1}, respectively). In contrast to [S]-5-MTHF, [S]-folinic acid is not bound to human serum albumin at physiological conditions, leading to a higher renal clearance of [S]-folinic acid [23]. The renal clearance of unbound [S]-5-MTHF was similar to that of [R]-5-MTHF and creatinine, indicating that active absorption of the isomers is unlikely. One can only speculate about the differences in the renal clearance of female and male subjects. We were

unable to correlate the different urine volume with the excreted amount of drug. In addition, the plasma protein binding of both isomers was equal for both genders.

The amount of drug excreted into urine was similar for both isomers. It must be noted, however, that after 24 h of sample collection low plasma levels of [R]-MTHF were observed. Thus, the amount of [R]-MTHF excreted into urine and the renal clearance appears to be slightly underestimated in this study. Based on the differences between the AUC and the AUC(0,24h) the error was estimated to be approximately 8% for [R]-MTHF and < 1% for [S]-MTHF.

Although the expansion of reduced folates in colon adenocarcinoma xenografts after administration of folinic acid has been investigated in detail [24, 25], only preliminary data concerning intratumoural folate levels after administration of folinic acid and 5-MTHF have been reported. In this study the administration of 200 mg/m^2 folinic acid or 5-MTHF resulted in a threefold expansion of $N^{5,10}$-methylene-tetrahydrofolate for both agents [26].

The systemic availability of physiological tetrahydrofolates after intravenous administration can be compared with the following assumptions: after administration of *rac*-folinic acid the AUC of circulating [S]-folate (AUC of the metabolite [S]-5-MTHF + AUC of [S]-folinic acid) is compared with the AUC of [S]-5-MTHF after administration of *rac*-5-MTHF (AUC standardised to 100 mg tetrahydrofolate/m^2: 2.14 *vs* 2.51 mmol l^{-1} min [27]; 2.52 *vs* 2.51 mmol l^{-1} min [22]; 3.24 *vs* 2.51 mmol l^{-1} min [3]; 3.34 *vs* 2.51 mmol l^{-1} min [2]).

To summarise our data, stereoselective mechanisms dominate the pharmacokinetics of *rac*-5-MTHF. Parallels to the pharmacokinetic behaviour of [R]-folinic acid and [S]-folinic acid after administration of *rac*-folinic acid can be drawn. In combination with 500 mg 5-FU/m^2 a single dose of up to 600 mg *rac*-5-MTHF/m^2 can safely be administered to patients with gastrointestinal cancer. Presuming a similar pharmacological activity of 5-MTHF and folinic acid from *in vitro* data, a pharmacokinetic comparison between the biologically active [S]-isomers indicates a similar availability in humans. A similar therapeutic benefit of 5-MTHF in the biochemical modulation of 5-FU is suggested by the comparison of some of the *in vitro* and *in vivo* data, but not by all investigations.

Recent findings established folic acid as an attractive alternative in the modulation of 5-FU [28]. In order to compare the efficacy of folic acid, folinic acid and 5-MTHF, prospectively randomised clinical trials are warranted.

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