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Disposition of 5-formyl- and 5-methyltetrahydrofolic acid in serum after i.v. bolus of calcium folinate: pharmacokinetic drug interaction with preadministered interferon-alpha-2b

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SUMMARY

The influence of interferon (IFN) coadministered subcutaneously with the biomodulating agent folinic acid on the blood serum levels of 5-formyl-tetrahydrofolic acid (CHO-THFA) and the biotransformation into its main metabolite in the blood 5-methyltetrahydrofolic acid (CH_3-THFA) was studied in patients receiving a chemotherapy with 5-fluorouracil. IFN causes a clear decrease of the serum concentrations of CHO-THFA and a statistically significant decrease of CH_3-THFA concentrations ($P < 0.01$). The effect on serum concentrations could be observed in each patient, but in a different order of magnitude. As a consequence, the preadministration of IFN leads to a significant change in the basic pharmacokinetic parameters of both compounds: the mean area under the concentration-time curve is decreased at 27.4% for CHO-THFA ($P < 0.025$) and at 22.4% for CH_3-THFA ($P < 0.025$), respectively. The total body clearance is elevated at 45.4% for CHO-THFA ($P < 0.05$) and at 23.4% for CH_3-THFA ($P < 0.05$). The mean volume of distribution is increased by IFN at 38.2% for CHO-THFA ($P < 0.025$) and at 22.3% for CH_3-THFA ($P < 0.05$). The nearly identical mean residence time in both groups indicates that CHO-THFA elimination is not affected by IFN. But the results prove a certain interaction between IFN and CHO-THFA. IFN accelerates the distribution of CHO-THFA as well as of its main metabolite from the blood into the tissue or activates the biotransformation of CHO-THFA into CH_3-THFA inside the cells of the tissue. The extent of biotransformation of CHO-THFA into CH_3-THFA, which takes place in the **blood**, is not influenced by IFN because percentage AUC-ratios CHO-THFA:CH_3-THFA were 89.5:10.5% for the control group and 88.8:11.2% for the IFN group.

INTRODUCTION

The biochemical modulation of anticancer agents is a new investigational approach to enhance their therapeutic effectiveness. The increase of the low response rate of 5-fluorouracil (5FU), 20–25% (1), as well as the improvement of its therapeutic efficacy by combination with biomodulating agents like 5-formyltetrahydrofolic acid (CHO-THFA) has been demonstrated recently (2–5). This effect is based on the augmentation of the therapeutic active metabolite of CHO-THFA, 5,10-methylenetetrahydrofolic acid, which is formed intracellularly and inhibits the proliferation of the tumor cell by complex formation with the enzyme thymidilate synthetase (6).

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The naturally occurring cytokine, interferon (IFN), has been shown to have cytotoxic and antiproliferative effects (7,8) and to enhance the 5FU effect on human colorectal cancer cell lines (9). Several Phase II studies have demonstrated an increase of the response rate of 5FU between 30–60% when IFN is coadministered (10). The double modulation of 5FU with folinic acid/IFN is considered to improve the tumoral response of 5FU in the treatment of colon adenocarcinoma (11,12). This triple combination, however, abrogates the statistically significant IFN effect on the pharmacokinetics of 5FU (13).

As we have shown, the combination of 5FU with IFN and dipyridamole (DPM) leads to a drastic change in the pharmacokinetics of 5FU, whereas the combination of folinic acid/DPM has no measurable effect on the 5FU kinetics (14). Analysis of serum and red blood cell samples revealed that the biotransformation of CHO-THFA into CH_3-THFA also takes place in red blood cells (a subcompartment of the blood) within a few minutes after administration of folinic acid (15–19).

While, at the present, no information is available about the serum concentrations of CHO-THFA and its biotransformation into CH_3-THFA when IFN is coadministered, the serum profile and the basic pharmacokinetic parameters of both compounds were evaluated under the influence of preadministered IFN.

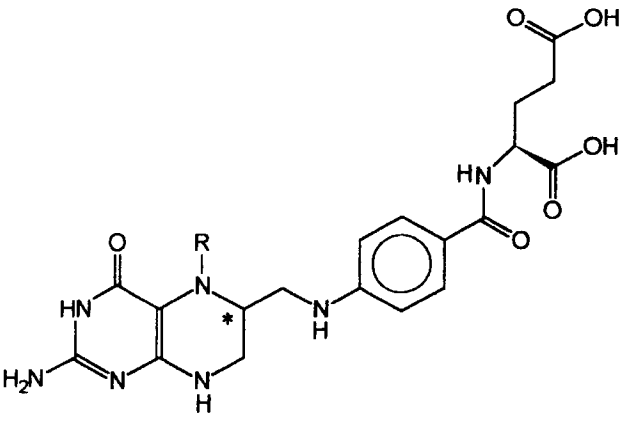

Fig. 1 : Chemical structure of CHO-THFA (R = –CHO) and CH_3-THFA (R = $-CH_3$).

MATERIALS AND METHODS

Drugs

Folinic acid (Leucovorin) was supplied by Cyanamid Lederle Pharmaceuticals as an aqueous solution for injection, containing 200 mg folinic acid calcium salt per 10 ml water. For chromatographic standards, CHO-THFA and CH_3-THFA were purchased from Sigma Chemical Company (St Louis, MO, USA). Recombinant human IFN-alpha-2b (Intron A) was supplied by Aesca (Traiskirchen, Austria; manufactured by Schering Plough Inc., NJ, USA). All chemicals were of analytical grade and all solvents were of HPLC grade purity (Merck, Darmstadt, Germany).

Patients and drug administration

The study was carried out as a prospective crossover design on 10 patients suffering from colorectal cancer and undergoing a weekly chemotherapy with 5FU.

Table I : Characteristics of patients.

Subject	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*Mean ± SEM*
Weight	84	83	66	62	64	76	62	62	72	64	60.4 ± 8.5
Height	165	165	153	156	182	180	164	179	160	176	168 ± 10.5
Age	67	62	55	56	52	67	63	64	66	67	61.9 ± 5.6
Sex	F	F	F	F	M	M	M	M	F	M	—
Dose LV	380	380	350	320	364	400	320	360	350	350	360 ± 29
Dose IFN	9	9	9	9	9	9	9	9	9	9	9 ± 0
mg/kg	4.52	4.58	5.30	5.16	5.69	5.26	5.16	5.81	4.86	5.47	5.18 ± 0.43
V_{blood}	4704	4773	4224	4030	4640	3914	4402	4340	4356	4448	4381 ± 279
V_{serum}	3108	3113	2772	2604	2880	3116	2697	2728	3024	2720	2876 ± 198

From each patient entering the trial, written permission was obtained after complete information about the scope of the study. Inclusion criteria were good performance status expressed by a Karnovsky index of > 70%, leucocyte count > 3500/ml, platelet count > 100.000/ml, maximum serum creatinine level of 1.5 mg/dl and a serum bilirubin level (2 mg%. Table I characterizes the individual data of the patients.

Folinic acid and IFN were administered during three consecutive cycles of chemotherapy with 5FU as follows:

1st cycle
5FU control cycle (750 mg/m^2 5FU was given as an intravenous short bolus).

2nd cycle
After a rest of 1 week, 5FU as in cycle 1 but with CHO-THFA (200 mg/m^2) given intravenously 5 min before 5FU.

3rd cycle
5FU and CHO-THFA as in cycle 3, but additionally 9 mU IFN 3 times weekly, subcutaneously for one week, before 3rd 5FU bolus.

Analytical approach

Blood samples (5 ml) were collected during cycles 2 and 3 (at the following times: 0, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min after administration) in EDTA-impregnated tubes containing 1 mg ascorbic acid (sodium salt) for drug stabilisation. Samples were vortexed for 1 min (Paramix, Julabo, Germany) and centrifuged for 5 min at 6000 rpm (Biofuge, Haereus Christ, Austria). Finally, 1 ml of serum was removed and stored at –30°C until analysis within 2 weeks to prevent degradation of the compounds in the samples.

Sample clean-up from the serum was performed by solid-phase extraction using C-18 cartridges (100 mg packing volume, 1 ml volume, Varian, Ann Harbor City, USA). The cartridges were washed with 3 ml of methanol and preconditioned with 2 ml of Pic A (424 mg/150 ml phosphate buffer, containing 347 mg NaH_2PO_4 and 1458 mg Na_2HPO_4). 1 ml of serum sample was sucked through the cartridge under slight vacuum (flow rate: 1 ml/min) and washed with 2 ml of Pic A solution. Finally, CHO-THFA and CH_3-THFA were eluted from the cartridge with 1 ml of methanol.

Quantitation of the compounds was performed by reversed-phase high performance liquid chromatography using ion-pair technique with Pic A as described (22,23).

Biometric data assessment

Pharmacokinetics of CHO-THFA and CH_3-THFA were calculated by use of a PC 486DX40 with the pharmacokinetic program PcNonlin 4.1. Serum concentration-time data for CHO-THFA were fitted according to an open two compartment model. CH_3-THFA kinetics, however, were calculated by a non-compartment model (24) using trapezoidal rule. The following pharmacokinetic parameters were calculated for CHO-THFA or CH_3-THFA, respectively, and checked for significance.

A:	intercept with y-axis in the alpha-phase (µg/ml)	CHO-THFA, –
B:	intercept with y-axis in the beta-phase (µg/ml)	CHO-THFA, –
C_{max}:	peak concentration (µg/ml)	CHO-THFA, –
AUC_{last}:	area under the concentration-time curve from 0 to 240 min (µg/ml.min)	CHO-THFA, CH_3-THFA
alpha-HL,:	half-life of alpha-phase (min)	CHO-THFA, –
beta-HL:	half-life of the beta-phase (min)	CHO-THFA, –
K10-HL-:	half-life of elimination (min)	CHO-THFA, –
MRT:	mean residence time (min)	CHO-THFA, CH_3-THFA
Vd:	volume of distribution (ml)	CHO-THFA, CH_3-THFA
Vss:	volume of distribution in steady-state (ml)	CHO-THFA, –
Cl_{tot}:	total body clearance (ml/min)	CHO-THFA, CH_3-THFA

Anova analysis of variance was performed using the scientific statistical modules Wistat and Statist on an Atari MegaST personal computer. The minimum level for significance was $P < 0.05$.

For additional information the amount of free

CHO-THFA (% of the dose) which is present in the blood immediately after administration was calculated using the following equation, where V_{serum} was obtained by a nomogram using sex, body weight and age of subjects as described (28).

$$\% \text{ Dose} = \frac{C_{max}\ (\mu g/ml) \cdot V_{serum}\ (ml) \cdot 100}{\text{Dose}\ (\mu g)} \qquad \text{Eq. 1}$$

RESULTS AND DISCUSSION

Serum concentrations

After i.v. administration of 200 mg/m^2 leucovorin, a biphasic concentration-time profile of CHO-THFA in the serum results with an initial distribution phase (15 min) followed by the phase of the terminal elimination from the central compartment. Table II compares the serum concentrations of CHO-THFA for each subject in both groups of the study.

As can he seen from Table II, the preadministration of IFN to folinic acid leads to lower serum concentrations of CHO-THFA in comparison with the control group (decrease of 10–27%). The analysis of variance revealed a statistically significant difference of serum concentrations for the samples collected at 20, 30, 90 and 120 min after administration ($P < 0.01$ to $P < 0.05$), but not for the other time points. This might be caused by a higher interindividual variability of the patients (e.g. subject J).

CH_3-THFA appears slowly in the serum with concentrations increasing from 0.53 ± 0.19 µg/ml (10 min) up to 2.09 ± 0.48 µg/ml (4 h after administration of folinic acid). When IFN is preadministered, the biotransformation of CHO-THFA into CH_3-THFA in the blood seems to be distinctly reduced, as the mean serum concentrations are lower than in the control group (from 0.31 ± 0.23 µg/ml at 10 min to 1.46 ± 0.54 µg/ml after 4 h). This IFN—CH_3-THFA drug interaction could be observed in each subject and was statistically significant over the whole investigated

Table II : Serum concentrations (µg/ml) of CHO-THFA with and without IFN.

Subject	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*Mean ± SEM*	*P*
Control group												
10	47.1	31.1	25.9	31.9	30.9	29.1	29.2	34.4	29.4	21.5	31.05 ± 6.64	NS
20	21.9	15.2	13.3	24.1	18.4	21.1	24.4	29.7	19.9	15.6	20.36 ± 4.99	A
30	19.2	12.0	11.6	15.1	16.1	19.3	23.4	25.2	16.8	13.5	17.22 ± 4.58	A
45	16.6	10.5	11.2	13.3	13.7	17.2	18.0	19.9	14.1	11.4	13.59 ± 4.80	NS
60	14.5	8.4	10.7	12.2	12.1	13.7	15.3	16.6	12.7	10.5	12.67 ± 2.45	NS
90	12.5	9.3*	8.2	10.1	10.4	13.2	13.4	13.5	10.3	9.9	11.08 ± 1.90	B
120	10.1	7.9	5.9	8.2	8.5	11.2	9.8	9.5	8.7	8.3	8.81 ± 1.45	C
180	7.6	5.7	5.1	6.5	4.6	9.2	7.2	6.9	6.8	6.3	6.59 ± 1.31	NS
240	5.1	4.8	3.0	5.7	4.3	7.7	5.8	4.5	4.4	5.1	5.04 ± 1.23	NS
With IFN												
10	26.2	14.2	28.3	22.8	24.4	28.8	26.9	39.5	33.7	9.2	25.40 ± 8.73	
20	14.1	8.2	17.2	16.4	16.3	27.5	19.6	20.9	24.1	3.4	16.77 ± 7.10	
30	12.2	6.3	14.3	12.8	10.7	25.0	17.3	17.7	17.7	2.4	13.64 ± 6.39	
45	11.4	5.4	14.8*	11.8	9.4	22.1	15.0	16.3	14.2	2.1	12.25 ± 6.58	
60	13.4*	5.2	13.4	10.3	7.3	19.5	12.5	14.8	10.8	0.9	10.81 ± 5.27	
90	9.9	4.4	12.1	7.3	6.0	12.7	11.4	9.6	9.2	0.6	8.32 ± 3.79	
120	7.8	2.2	6.7	5.1	6.1*	10.9	9.2	7.6	8.4	0.2	6.42 ±3.22	
180	6.6	1.3	6.4	4.4	5.4	9.3	6.4	6.9	7.3	<0.01*	5.40 ± 2.82	
240	6.4	0.8	2.7	1.6	4.7	8.7	5.7	5.1	4.8	<0.01*	4.05 ± 2.72	

*Value does not fit to the concentration-time curve. Values < 0.01 are at a concentration beyond the limit of detection of 0.01 µg/ml.
P level of probability between control group and IFN group: NS not significant, A = $P < 0.050$, B = $P < 0.025$, C = $P < 0.010$.

Table III: Serum concentrations (µg/ml) of CH_3-THFA with and without IFN.

Subject	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*Mean ± SEM*	*P*
Control group												
10	0.59	0.81	0.24	0.60	0.73	0.58	0.48	0.47	0.22	0.62	0.53 ± 0.19	B
20	1.03	1.01	0.26	0.72	0.92	0.80	0.59	0.62	0.31	0.64	0.69 ± 0.26	D
30	1.29	1.12	0.71	0.99	1.08	0.98	0.64	0.89	0.59	0.66	0.90 ± 0.24	C
45	1.51	1.33	0.95	1.17	1.39	1.19	0.50	1.09	0.78	0.77	1.00 ± 0.39	A
60	1.94	1.52	1.08	1.48	1.72	1.39	1.36	1.37	0.89	0.81	1.36 ± 0.35	B
90	1.97	1.50*	1.27	1.78	1.88	1.67	1.79	1.63	1.15	0.88	1.55 ± 0.35	A
120	2.01	1.74	1.42	1.91	2.05	1.81	1.90	1.66	1.26	0.93	1.67 ± 0.36	B
180	2.00*	1.98	1.59	2.18	2.22	2.24*	1.96	1.78	1.42	0.98	1.84 ± 0.40	D
240	2.33	2.16	1.89	2.59	2.79	2.22	2.17	2.01	1.69	1.07	2.09 ± 0.48	D
With IFN												
10	0.32	0.10	0.22	0.31	0.19	0.53	0.14	0.52	<0.01	0.77	0.31 ± 0.23	
20	0.59	0.19	0.37	0.36	0.48	0.68	0.46	0.55	0.29	0.32	0.43 ± 0.15	
30	0.81	0.44	0.63	0.38	0.67	1.10	0.55	0.65	0.58	< 0.01	0.58 ± 0.28	
45	0.90	0.63	0.88	0.66	0.91	1.17	0.84	0.77	0.72	< 0.01	0.75 ± 0.30	
60	1.04	0.82	1.33	0.81	1.18	1.22	1.13	1.06	1.21	< 0.01	0.98 ± 0.38	
90	1.27	1.26	1.98	1.11	1.54	1.25	1.23	1.20	1.09*	< 0.01	1.19 ± 0.49	
120	1.31	1.40	1.81*	0.82*	1.71	1.59	1.46	1.20*	1.29	< 0.01	1.26 ± 0.52	
180	1.49	1.38*	2.01	1.17	1.89	1.65	1.51	1.27	1.36	< 0.01	1.37 ± 0.54	
240	1.72	1.56	1.90	1.44	1.89*	1.68	1.64	1.36	1.45	< 0.01	1.46 ± 0.54	

*Value does not fit to the concentration-time curve. Values < 0.01 are at a concentration beyond the limit of detection of 0.01 µg/ml.
P level of probability between control group and IFN group: NS not significant, A = $P < 0.050$, B = $P < 0.025$, C = $P < 0.010$, D = $P < 0.005$.

time period (*P* ranges from 0.05–0.005, compare Table III).

The concentration-time profiles are very similar for all subjects with the exception of patient J, which shows mostly undetectable amounts of CH_3-THFA in the IFN group. Obviously, the IFN effect is pronounced in this patient. The data, however, were not excluded from the biometric calculations because, on the one hand the concentrations in the control group did not differ anyhow from the other subjects, and on the other hand the clinical evidence did not show increased renal or liver impairment or main disorders in the haemograms.

Pharmacokinetic parameters

For CHO-THFA, the pharmacokinetic parameters were derived using an intravasal open two-compartment model; for CH_3-THFA, however, only a noncompartmental model could be used due to its formation from CHO-THFA (24). Table IV compares the pharmacokinetic parameters of CHO-THFA of the control and the IFN group.

The pharmacokinetic profile of CHO-THFA is in accordance with previous findings (25–27) with a mean initial distribution phase of about 10 min and a mean half life of the terminal elimination of 130 min. The noncompartmental calculation of the MRT reveals nearly identical values, too: 159 ± 42.7 min for the control and 151.1 ± 87.2 min for the IFN group. Both calculated parameters prove that the half-life of terminal elimination is not influenced by IFN, so drug elimination is not the reason for lower serum concentrations of CHO-THFA.

The average intercepts of the concentration-time curves for the alpha and beta phase are 87 µg/ml for the hybrid constant A and 16.7 µg/ml for B. The sig-

Table IV : Pharmacokinetic parameters of CHO-THFA with and without IFN.

Subject	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*Mean ± SEM*	*P*
Control group												
A	133.4	96.8	92.4	35.5	81.4	25.3	17.5	21.5	44.4	23.3	87.1 ± 101.6	NS
B	21.6	12.3	14.0	13.9	18.9	18.1	16.1	20.1	18.1	13.6	16.7 ± 3.1	A
AUC	4701	3199	2842	3711	2987	4862	3955	3634	3153	3093	3614 ± 708	B
K10-HL	9	20	10	52	21	77	82	61	35	58	42.5 ± 27.1	NS
alpha-HL	3	4	3	10	4	9	26	21	5	6	9.1 ± 8.0	NS
beta-HL	108	146	107	158	94	174	141	103	108	147	128.6 ± 27.7	NS
Vd	1070	3481	1599	6475	3632	9212	9522	8667	5606	9749	5901 ± 3330	NS
C_{max}	155	109	106	49	100	43	34	42	62	37	103.7 ± 102.8	NS
Cl	81	119	116	86	122	82	81	99	111	116	101.3 ± 17.3	A
MRT	113	173	117	198	117	236	176	127	140	197	159.4 ± 42.7	NS
V_{ss}	9171	20520	13617	17058	14199	19376	14261	12587	15510	22962	15926 ± 4107	B
With IFN												
A	78.9	43.3	98.8	26.0	33.8	30.6	23.7	48.8	37.9	4.5	52.7 ±43.7	
B	12.6	8.7	18.3	16.2	7.6	2.2	18.3	20.8	12.4	9.9	12.7 ± 5.8	
AUC	3769	1014	3044	1979	3317	2120	3360	3518	3817	309	2625 ± 1218	
K10-HL	29	14	15	32	56	45	55	15	53	15	33.2 ± 17.7	
alpha-HL	4	4	3	5	10	60	7	3	12	15	12.3 ± 17.3	
beta-HL	182	63	98	76	258	163	118	93	177	15	124.3 ± 70.8	
Vd	4149	7298	2817	7818	8797	12228	7622	2123	6969	24256	8409 ± 6316	
C_{max}	92	52	117	42	41	33	42	70	50	14	64.3 ± 47.3	
Cl	101	375	108	167	110	189	95	102	92	1134	147.2 ± 86.3	
MRT	232	72	122	99	321	163	159	107	215	21	151.1 ± 87.2	
V_{ss}	23359	26953	13176	16506	35237	30804	15168	10963	19689	25263	22012 ± 7657	

P level of probability between control group and IFN group: NS not significant, A = $P < 0.050$, B = $P < 0.025$.

nificant difference of B ($P < 0.05$) is rather surprising, as this could indicate a change in drug elimination. The addition of A and B to C_{max} amounts to 103.7 ± 102.8 µg/ml in the control group and decreases by 38% to 64.3 ± 47.3 µg/ml. But due to the high standard deviation in the control group, a statistical difference was not calculable.

The large increases in the volume of distribution of 42% and of the total serum clearance of 45% ($P < 0.025$), however, confirm a possible change of drug distribution under the influence of IFN: either an accelerated distribution from the central to the deep compartment or an inhibition of redistribution from the deep to the central compartment.

Further information can be obtained from the total body clearance, which is accelerated by about 45% by IFN for CHO-THFA and for CH_3-THFA the clearance is accelerated by 23%. This accelerated total clearance from the blood indicates a higher distribution rate for the compounds from serum into tissue.

For CH_3-THFA a clear influence of IFN can be observed in the case of total clearance and volume of distribution, which are increased by IFN significantly ($P < 0.05$). In accordance with CHO-THFA, the mean residence time is not affected by IFN (compare with Table V).

The most important parameter expressing the bioavailability of a drug in the blood is the area under the concentration-time curve. A statistically significant difference can be calculated for AUC_{last}, which is diminished by 27.4% for CHO-THFA and by 22.4% for CH_3-THFA when IFN is coadministered ($P <$

Table V: Pharmacokinetic parameters of CH_3-THFA with and without IFN.

Subject	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*Mean ± SEM*	*P*
Control group												
AUC	435	390	305	422	452	405	381	358	271	207	378.9 ± 60.0	B
MRT	135	139	145	144	142	142	143	140	146	134	141.8 ± 13.3	NS
Cl	873	975	917	759	806	987	840	1006	1294	1691	1014.8 ± 280	A
V_{ss}	118	135	133	109	114	140	120	141	189	227	142.6 ± 37.0	A
With IFN												
AUC	293	266	374	222	348	327	293	259	264	281	293.9 ± 48.1	
MRT	142	146	142	145	144	137	143	137	142	144	142.0 ± 16.1	
Cl	1297	1428	82	1441	1046	1223	1092	1390	1325	1400	1252.4 ± 189	
V_{ss}	183	209	125	209	150	168	155	190	186	170	174.5 ± 26.5	

P level of probability between control group and IFN group: NS not significant, A = $P < 0.050$, B = $P < 0.025$.

0.025). This signifies that the systemic circulation of both compounds is reduced at about 25%. As the dimension of the decrease of the AUC values is nearly identical for CHO-THFA and CH_3-THFA, IFN obviously has no influence on the biotransformation of CHO-THFA into CH_3-THFA **in the blood**. For some drugs it has been reported that IFN may reduce the hepatic metabolism through a reduction in the level of microsomal cytochrome P-450, e.g. antipyrene (20) or theophylline (21).

The bioavailability of the compounds (expressed as percentage AUC values) amounts to 89.5% for CHO-THFA and 10.5% for CH_3-THFA in the control-group (factor = 8.5). A very similar percentage distribution results in the IFN group: 88.8% for CHO-THFA and 11.2% for CH_3-THFA (factor = 7.9). This equal ratio signifies that drug-distribution from the blood into tissue predominates when IFN is preadministered.

Figure 2 depicts the amount of unbound CHO-THFA (in % of the administered dose) which is available in the serum immediately (t = 0 min) after i.v. administration of folinic acid. (These data can be derived from the C_{max} and the serum volume of the patient as given in the experimental section.)

This mean percentage of CHO-THFA amounts to 59.6 ± 33.2% of the dose for the control group and 45.7 ± 24.1% for the IFN group (this is a reduction of about 24%). Due to high standard deviations (especially subject X), this observation is not significant. Yet, a very surprising observation could be made: the collective consisted of 5 female and 5 male individuals and there was a statistical difference with regard to the sex. In the control group as well as in the IFN group, the amount of CHO-THFA was higher for female patients: 78.6 ± 33.5% for female and 40.6 ± 21.6% for male patients (this is 59% more for female, $P < 0.033$) in the control group.

In the IFN group, the findings were very similar: 57.6 ± 15.2% for female and 33.8 ± 17.9% for male patients (*see* Fig. 2). Whether this is an incidental finding or not cannot be explained at the present as the

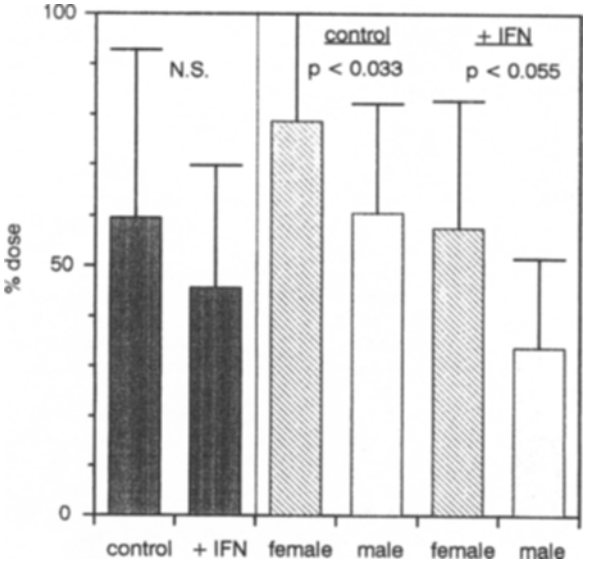

Fig. 2: Amounts of free CHO-THFA in serum (% of administered dose) at t = 0 min with and without IFN.

comparison is only based on 5:5 patients and from the hemograms (representing, e.g. metabolic capacity) no information could be obtained.

CONCLUSIONS

In several pharmacokinetic studies, it has been demonstrated that a drug interaction between IFN and fluorouracil is considerable. The triple combination IFN + fluorouracil + folinic acid, however, abrogates this drug interaction. In the present study, we could demonstrate that there exists a drug interaction between the biomodulating agents IFN and CHO-THFA, too. When IFN is coadministered to folinic acid (as a modifying system for 5FU), lower serum concentrations of CHO-THFA and its main metabolite in the blood, CH_3-THFA, can be observed. Pharmacokinetic data assessment revealed no statistically significant difference for the terminal elimination half-life. But AUC values (representing an important indicator for bioavailability) are diminished and the total body clearance was accelerated significantly by IFN. An accelerated distribution of CHO-THFA from the blood into the tissue might be one possible reason for this observation. Biotransformation of CHO-THFA into CH_3-THFA within the blood seems to be reduced by IFN. The present results give evidence that IFN causes a (desired) reduction of the systemic circulation of CHO-THFA in the blood and produces, as a consequence, higher tissue levels. By that means the formation of the therapeutically active 5,10-methylene-THFA should be promoted.

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