Preliminary study on pharmacokinetics of dacarbazine and fotemustine in glioblastoma multiforme patients does not indicate gender-specific differences

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Twelve patients (six female and six male) with histologically proven glioblastoma multiforme were investigated during the administration of the first cycle of dacarbazine (D; 200 mg/m^2) and fotemustine (F; 100 mg/m^2). In total, 18 blood samples were collected for pharmacokinetic analysis (maximum plasma concentration, area under the concentration–time curve and total clearance) of D and F at 14 time points during therapy. D, its metabolite 5-aminoimidazole-4-carboxamide and F were evaluated by reversed-phase HPLC. For statistical calculations, groups were compared by the non-parametric Wilcoxon test. $p<0.05$ was considered statistically significant. No significant gender-dependent differences were observed in the pharmacokinetics of D and F. An additional response re-evaluation of 100 patients (50 female and 50 male) with glioblastoma multiforme, treated at our institution with D and F, gave no hint of any gender-dependent different response rates. We conclude that there is no evidence, neither from pharmacokinetic nor from our clinical data, to consider different dosages of D and F in female and male patients with glioblastoma multiforme. *Anti-Cancer Drugs* 15:495–498

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Introduction

Little is known about gender-specific differences in pharmacokinetics and pharmacodynamics for chemotherapeutic agents. An exclusive exception is 5-flourouracil (5-FU), as a 10% increased 5-year survival rate for women as compared to men after adjuvant 5-FU-based chemotherapy for colon cancer has recently been published in the *New England Journal of Medicine* [1]. Concerning toxicity, recent data confirmed earlier findings that women experience toxicity more frequently and with more severity than men after administration of a 5-day bolus schedule [2–6].

A gender-related difference in the pharmacokinetics was postulated for doxorubicin and for epirubicin [7,8], and such a difference between pharmacokinetics and hematotoxicity in males and females has been reported for fotemustine (F) [9–11].

Concerning dacarbazine (D), inconclusive data have been reported with respect to gender-related differences in response to chemotherapy. While a better response to D among women has been reported in some studies [12,13], it has not been confirmed in other trials [14,15]. A striking gender-dependent difference in response rates following D administration (50% in women as compared to 12% in men) was reported for malignant melanoma and remains to be confirmed [12]. Thus far, pharmacokinetic studies on sex-based differences for D have not yet been performed. Due to the fact that the combination of D and F is widely administered to patients with malignant gliomas [16,17] and malignant melanomas, we investigated the possible existence of gender-specific differences with regard to both substances.

Methods

The study was performed after approval by the local ethics committee. All patients volunteered to participate in the pharmacokinetic study and gave their written informed consent before start of chemotherapy.

Patient characteristics

Twelve patients (six male and six female) patients with histologically proven glioblastoma multiforme were investigated during the administration of the first cycle of D and F.

Median age was 47.5 (range 26–60) years. Median body surface area was 1.83 (range 1.53–2.11) m^2. Administered median total dose of D was 367 (range 306–422) mg and of F was 183 (range 153–211) mg.

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Six patients (three male and three female) were administered anticonvulsants. Two patients received lamotrigin in daily dosages of 75 and 100 mg, respectively. Four patients were administered carbamazepine in daily dosages between 400 and 900 mg.

Inclusion criteria

Patients with newly diagnosed and histologically proven glioblastoma multiforme basing on the WHO classification [18] were eligible for study inclusion. Patients had to be aged 20–60 years, have a Karnofsky performance score $> 60\%$ and a life expectancy of > 8 weeks.

For chemotherapy application, patients were required to have adequate liver, renal and bone marrow function. Pregnant or nursing women as well as patients with acute infections were not eligible. Adequate contraception was mandatory. Patients were not allowed to be under cytotoxic chemotherapy due to concurrent malignancy and were not allowed to have had any other cytotoxic chemotherapy within half a year before study entry. Intake of hydrocortisone was not allowed due to a described influence onto the pharmacokinetic of D. Patients body weight had to be within a range of $> 30\%$ above and $< 15\%$ below normal weight according to evaluation with body mass index.

Therapeutic protocol

Chemotherapy consisted of D at a dosage of 200 mg/m^2 (diluted in 250 ml normal saline) and F at a dosage of 100 mg/m^2 (diluted in 250 ml glucose 5%). Both solutions were protected from light and were given i.v. in an outpatient setting. D was administered over 30 min; 30 min after termination of D infusion, F was given over 60 min.Both solutions were infused via a MGVG IP85 volumetric pump (Braun, Munich, Germany) into a large peripheral vein.

Premedication consisted of dexamethasone 4 mg and tropisetron 8 mg diluted in 250 ml isotonic sodium chloride. Tropisetron was orally administered for 2 consecutive days after chemotherapy application.

Pharmacokinetics

In total, 18 blood samples were collected for the evaluation of D and F at 14 time points during therapy. Blood samples were drawn immediately before start of D infusion ($t = 0$) as well as 15 and 30 min after D infusion start (= end of D infusion). To assess the concentration–time profile of D, further samples were collected after 40, 50, 60, 90, 120, 195, 255 and 315 min. At $t = 60$ min the F infusion was started. To assess this drug, samples were collected at $t = 90$ and 120 min (= end of F infusion) followed by samples collected after 135, 165, 195 and 255 min.

Blood samples were drawn from a vein in the arm opposite to that used for D and F infusion.

D blood samples were placed into light-protected tubes containing EDTA as anticoagulant. Plasma was immediately separated by centrifugation at 3000 r.p.m. for 5 min at 4°C, transferred to amber glass vials, shock-frozen in a mixture of ethanol/dry ice and stored at –80°C until analysis.

For F analysis, blood samples were collected in light-protected tubes containing EDTA as anticoagulant. Plasma was immediately separated by centrifugation at 3000 r.p.m. for 5 min at 4°C. Then, 1 ml of plasma was transferred into amber glass vials together with 0.4 ml 0.1 M citric acid for stabilization; thereafter they were shock-frozen in a mixture of ethanol/dry ice and stored at –80°C until analysis.

D and its metabolite 5-aminoimidazole-4-carboxamide (AIC) were evaluated by reversed-phase HPLC using a recently established method [19]. F was assessed after solid-phase extraction by reversed-phase HPLC as originally described [20].

The quality control of the analytical methods was reproduced with the following results. The detection limit was 0.5 μg/ml plasma for D and AIC. The standard curve was linear up to 200 μg/ml plasma (coefficient of correlation $r = 0.999$). The intra-assay coefficient of variation for D and AIC ranged from 6.2 to 7.3% (low calibrator with 1 μg/ml plasma, $n = 5$ injections) and from 1.0 to 2.0% (high calibrator with 100 μg/ml plasma). Considering F, the detection limit was 0.2 μg/ml plasma. The standard curve was linear up to 50 μg/ml plasma (coefficient of correlation $r = 1.0$). The intra-assay coefficient of variation of F ranged from 0.5 to 2.0% (calibrators with 50 μg/ml plasma and 1 μg/ml, respectively; $n = 5$ injections).

Pharmacokinetic data analysis

Pharmacokinetic data were analyzed using Kinetica 2000 (Innaphase, Philadelphia, PA) to calculate the area under the concentration–time curve (AUC) Total clearance (CL_{tot}) was calculated by the ratio dose:AUC. For statistical calculations, groups were compared by the non-parametric Wilcoxon test.

$p < 0.05$ was considered statistically significant. Statistical evaluations were performed with the SPSS version 10.7 programme package.

Results

Pharmacokinetics

No statistically significant differences were observed considering the parameters AUC, maximum plasma

concentration and total clearance (Table 1). There was a slight trend in C_{max} of the metabolite AIC to be higher in males than in females ($p < 0.06$), but this did not reach statistical significance.

Additionally, the intake of anticonvulsants (lamotrigin, carbamazepine) did not influence the pharmacokinetic data of the investigated drugs.

Furthermore, we evaluated hematotoxicity and nausea/vomiting during the first cycle of chemotherapy according to the NCI common toxicity criteria [21]. We could not identify differences between female and male patients.

Response evaluation in the 12 study patients was performed after the second application of D and F, and based on MacDonald's criteria [22]. No gender-dependent differences were observed.

An additional response re-evaluation of 100 patients (50 female and 50 male) with malignant gliomas treated at our institution with D and F gave no hint of a gender-dependent response rates.

Discussion

No gender-related differences were found in both investigated substances, neither in D and its metabolite AIC, nor in F in patients with glioblastoma multiforme. This is in contrast to the observed difference in response rates between women (50%) and men (12%) reported for patients with malignant melanoma after therapy with D in combination with cisplatin [12]. Although Luger *et al.* [12] failed to exclude an inadvertent selection bias, the phenomenon of gender-dependent different response rates has remained unexplained. In particular, pharmacokinetic studies have not been performed so far.

Because D in combination with F has recently been established as an interesting first- and second-line therapy for glioblastoma multiforme [16,17], we focused on the gender-specific pharmacokinetic behavior of both substances; however, we were unable to identify gender-dependent differences in our study.

The data from our exploratory study provide an estimate for the difference, which has to be expected when considering pharmacokinetic key parameters such as AUC, maximum plasma concentrations and total clearance. Although gender-specific differences in the pharmacokinetics (if they exist) would only become evident in a population pharmacokinetics study, there are, however, examples from the literature where small numbers of patients were sufficient to unravel gender-specific differences such as the biochemical modulation of 5-FU [23]. In this present study, a total of 18 patients showed a highly significant difference in the total clearance of the modulator 5-methyltetrahydrofolate

Table 1 Pharmacokinetic parameters of D, AIC and F

Parameter	Female (no. of patients)						Male (no. of patients)					
	1	2[a]	3[b]	4	5	6[b]	1	2	3[a]	4	5[b]	6[b]
D												
C_{max}	3.75	4.51	8.08	3.47	3.29	3.88	12.21	4.75	5.65	8.34	3.90	2.77
MV; SD	4.50; 1.81						6.27; 3.47					
p												
AUC	4.37	4.79	6.58	4.64	3.50	5.45	8.55	4.15	4.18	5.79	3.35	3.11
MV; SD	4.89; 1.04						4.86; 2.04					
p												
CL_{tot}	75.1	73.1	53.8	67.6	105.6	56.1	47.0	101.6	87.1	66.6	121.2	124.7
MV; SD	71.88; 18.67						91.37; 30.68					
p												
AIC												
C_{max}	2.06	1.64	2.60	1.66	1.10	1.52	2.24	3.13	3.00	2.02	2.22	1.89
MV; SD	1.76; 0.51						2.42; 0.52					
p												
AUC	4.49	4.27	4.66	3.16	3.85	3.57	4.64	6.88	2.58	4.56	3.83	3.54
MV; SD	4.00; 0.58						4.34; 1.46					
p												
F												
C_{max}	1.68	1.15	1.42	1.53	2.11	1.11	3.28	1.76	1.66	1.72	1.18	0.63
MV; SD	1.50; 0.37						1.71; 0.89					
p												
AUC	1.73	1.14	2.12	1.64	2.54	1.00	3.01	2.10	1.78	2.09	1.43	0.59
MV; SD	1.70; 0.58						1.83; 0.80					
p												
CL_{tot}	95.1	153.1	83.6	95.7	72.9	153.6	66.7	100.7	102.3	92.5	142.4	329.7
MV; SD	109.00; 35.36						139.05; 96.52					
p												

[a]Patients receiving the anticonvulsant lamotrigin.
[b]Patients receiving the anticonvulsant carbamazepine. MV, mean value; SD, standard deviation; C_{max}, maximum plasma concentration (μg/ml plasma); AUC, area under the concentration–time curve (mg /l·h); CL_{tot}, total clearance (l/h). Statistical probability *p* not calculated, because of negligible differences to unfitted data.

comparing male and female patients with advanced colorectal cancer. With this number of patients, a statistical significance of $p < 0.05$ with a β error of < 0.10 is achieved in case of a mean pharmacokinetic difference of 50% with a pooled standard deviation of 30%. The standard deviation of 30% is in accordance with that described in the literature for AUC, and clearance of D and F [10,11,24,25]. Therefore, our current study was terminated after having analyzed 12 patients, because for clinical daily practice only differences exceeding the pharmacokinetic variability generally observed after i.v. administration of D and F, i.e. 25–35% for the parameters AUC and clearance, would demand dose adjustment in men and women.

Additionally, the pharmacokinetic data of D and F are in accordance with a re-evaluation of response rates in 100 patients with glioblastoma multiforme treated at our institution with both substances [16,17], which gave no hint of any gender-related differences.

Summarizing, there is no evidence, neither from pharmacokinetic nor from clinical data, to consider different dosages of D and F in female and male patients with glioblastoma multiforme. Nevertheless, a word of caution should be included because our results are based on a small group of patients with glioblastoma multiforme. Results may differ in patients with other tumor entities.

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