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Evaluation of the bioequivalence of tablets and capsules containing the novel anticancer agent R115777 (Zarnestra) in patients with advanced solid tumors

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SUMMARY

R115777 (Zarnestra) is a novel anticancer agent, currently undergoing phase III clinical testing. An open, cross-over trial was performed in 24 patients with solid tumors to compare the bioavailability of a new tablet formulation with the standard capsule formulation. Both dosage forms were administered once daily in doses of 300 or 400 mg. Patients received R115777 as a capsule on day 1 and as a tablet on day 2, or vice versa. Blood samples were drawn up to 24 hours after drug intake and R115777 levels were measured using a validated high performance liquid chromatography (HPLC) method. The following pharmacokinetic parameters were determined and compared for the two formulations: time to maximal plasma concentration (T_{max}), half-life ($t_{½}$), maximal plasma concentration (C_{max}) and area under the curve at twenty-four hours (AUC_{24h}). For the latter two parameters, 90% classical confidence intervals of the ratio tablet/capsule were calculated after a log-transformation, using an Analysis of Variance (ANOVA). For $t_{½}$ and T_{max}, no statistically significant differences were found between tablet and capsule. The point estimates of the ratio's of the log-normalized C_{max} and AUC_{24h} were 0.94 and 0.92, respectively, and the 90% confidence intervals were 0.81-1.09 and 0.83-1.03, which is within the critical range for bioequivalence of 0.80-1.25. In conclusion, the established pharmacokinetic parameters demonstrate that the capsule and tablet formulations of R115777 are interchangeable.

INTRODUCTION

R115777 (Zarnestra), (fig 1) is a novel anticancer agent, belonging to the class of farnesyltransferase inhibitors (FTIs). It is currently undergoing phase III clinical evaluation as single agent in advanced colon and pancreatic cancer. FTIs exert their antineoplastic activity by modulating cell biochemistry. In brief, they inhibit the enzyme farnesyltransferase, which is required for the maturation of several proteins, including Ras and Rho (1-3). Ras and Rho play a role in transducing stimulatory growth signals from the extracellular environment to the

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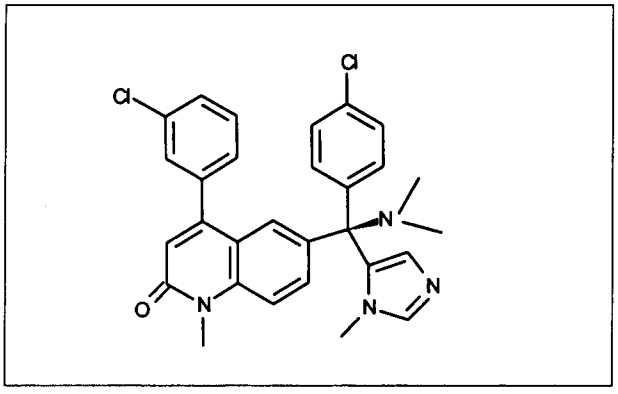

Fig. 1: Structural formula of R115777

cell nucleus. Ras is mutated in approximately 25% of all human malignancies, with the highest frequencies found in pancreatic (90%), colorectal (50%), and non-small cell lung (30%) tumors (4,5). This mutated Ras causes an overstimulation of cell proliferation, and can thus contribute to tumorgrowth (6,7). FTIs block these proteins and have demonstrated strong antineoplastic activity in both in vitro and in vivo studies (8-11). With R115777, several phase I trials have been completed, and even though the primary goal of such trials is not to evaluate antitumor efficacy, a number of tumor responses have been observed in patients with lung, pancreatic, cervix and colorectal carcinomas (12,13). We performed two phase I dose-escalating trials with continuous and intermittent daily oral R115777 in patients with advanced solid tumors. In these studies, R115777 was supplied as 100 mg capsules. At the highest dose levels, patients had to take ≥4 capsules twice a day. Hence, a more practical tablet formulation was prepared, also to conveniently dose patients in future studies. Here, we present the results of an open, crossover, pharmacokinetic study with R115777 in cancer patients, with the aim to compare the bioavailability of 100 mg tablets and the standard 100 mg capsules. The bioequivalence study was implemented in the ongoing phase I trials.

Patients and methods

Patient population

Patient inclusion criteria were derived from phase I clinical trials with R115777 also performed at the two participating Institutes: The Antoni van Leeuwenhoek Hospital in Amsterdam, the Netherlands, and the Fox Chase Cancer Center in Philadelphia, USA. Patients were eligible if they had a histologically confirmed diagnosis of a solid malignant tumor not amenable to established forms of effective therapy. Other eligibility criteria included a good performance status, anticipated life expectancy of at least 3 months and age ≥ 18 years. Previous anticancer chemotherapy had to be discontinued for at least four weeks before entry into the study, or six weeks in case of pretreatment with nitrosourea or mitomycin C. Radiation therapy should have ended at least four weeks prior to study entry. All patients had to have acceptable bone marrow function, white blood cells (WBC) > 3.500/μL and platelets > 100.000/μL; serum bilirubin within normal range, ASAT and ALAT ≤ 2 x normal upper limit or ≤ 5 x normal upper limit in case of hepatic metastases. The Medical Ethics Committees of the cooperating hospitals approved the study protocol, and all patients gave written informed consent.

Treatment plan and study design

R115777 tablets (100 mg) and capsules (100 mg) were supplied by Janssen Research Foundation. The colored hard gelatine capsules contained R115777, sugar spheres, hypromellose and macrogol and the tablets contained R115777, lactose, unmodified maize starch, hypromellose, microcrystalline cellulose, crospovidone, colloidial silicon dioxide and magnesium stearate. The drug was given at a dose of 400 or 300 mg qd, at least one hour after a meal. Patients were assigned to one of two dosing schemes: scheme A administered R115777 on day 1 as capsules and on day 2 as tablets, and scheme B vice versa.

Pharmacokinetics

Plasma concentrations of R115777 were determined by a validated high-performance liquid chromatographic (HPLC) method as described previously (12). On days 1 and 2 a pre-dose blood sample was drawn immediately prior to administration of study medication. Additional blood samples for the determination of R115777 plasma concentrations were drawn at 1, 2, 3, 5, 8 and 12 hours after drug intake. At least 6 mL of heparinized blood was collected for each sample. Samples were immediately placed on ice and centrifuged within two hours after collection (10 minutes, at approximately 1000 g or 2500 rpm). Separated plasma was transferred to polyethylene tubes, frozen by immersion in a dry ice/ethanol bath and stored at least at -20°C for subsequent drug analysis. Descriptive statistics were used to calculate the plasma concentrations of R115777 at each sampling time and for its pharmacokinetic parameters. The primary pharmacokinetic parameters C_{max}, and AUC_{24h} were analyzed on the linear and on the logarithmic scale, whereas the parameters T_{max} and $t_{½}$ were analyzed on the linear

Table 1: Individual pharmacokinetic parameters for R115777 per patient.

Patient	Dose (mg/day)	Schedule*	Capsule					Tablet					Tablet/Capsule		
			T_{max} (h)	C_{max} (ng/mL)	AUC_{12h} (ng*h/mL)	AUC_{24h} (ng*h/mL)	$t_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{12h} (ng*h/mL)	AUC_{24h} (ng*h/mL)	$t_{½}$ (h)	Ratio C_{max}	Ratio AUC_{12h}	Ratio AUC_{24h}
1	400	A	3.0	2597	11516	13068	2.8	1.0	1286	7335	8339	3.5	0.50	0.64	0.64
2	300	B	3.0	663	2993	3266	2.8	3.0	405	2407	2656	2.6	0.61	0.80	0.81
3	300	A	3.0	892	5440	6503	3.1	2.1	967	5035	5780	2.8	1.08	0.93	0.89
4	300	B	2.1	1337	5912	6516	2.5	3.0	1347	6148	6684	2.3	1.01	1.04	1.03
5	300	A	3.0	1417	6134	6544	2.3	3.0	800	2892	3135	2.2	0.56	0.47	0.48
6#	300	B	3.1	1222	6611	7115	1.9	2.0	339	1962	2069	2.1	0.28	0.30	0.29
7	300	A	3.2	1673	9692	11353	3.3	3.0	1801	11079	13322	3.5	1.08	1.14	1.17
8	300	B	2.0	994	3686	3988	2.1	1.0	1156	3700	3890	1.9	1.16	1.00	0.98
9	300	A	3.0	558	2428	2623	2.1	3.0	501	2895	3208	2.4	0.90	1.19	1.22
10	300	B	3.2	481	1759	1819	1.7	2.0	465	1499	1527	1.4	0.97	0.85	0.84
11	300	A	1.0	1778	6795	7326	2.4	1.0	2076	6598	7850	2.8	1.17	0.97	1.07
12	300	B	3.0	856	4526	5299	2.5	3.1	1181	5127	5685	2.6	1.38	1.13	1.07
13	400	A	2.1	1817	7409	8448	3.0	3.0	1403	7502	8712	3.3	0.77	1.01	1.03
14	400	B	1.1	2374	7395	7798	2.5	1.0	2037	6719	7177	1.7	0.86	0.91	0.9
15	400	B	3.0	2154	12098	13464	3.6	3.0	2467	11661	12954	2.9	1.15	0.96	0.96
16	400	B	3.0	1940	11644	12958	2.8	3.0	3907	16578	17489	2.4	2.01	1.42	1.35
17	400	A	2.0	925	3808	4115	2.7	3.0	833	3995	4288	2.7	0.90	1.05	1.04
18	400	A	2.0	4932	22638	23591	2.1	3.0	4718	19922	20669	2.1	0.96	0.88	0.88
19	400	A	5.0	888	5458	6480	3.7	5.0	1168	6983	8211	5.7	1.32	1.28	1.27
20	400	B	2.0	1243	4686	5701	4.5	2.0	378	1750	2354	3.2	0.30	0.37	0.41
21	400	B	5.0	844	5868	7530	4.7	5.0	681	5775	6957	3.1	0.81	0.98	0.92
22	400	A	3.0	1060	4901	5209	2.5	2.0	1257	5505	5922	2.7	1.19	1.12	1.1
23	400	B	3.0	1130	4905	5253	2.4	3.0	2031	6439	6657	2.0	1.80	1.31	1.27
24	400	B	5.0	491	3454	4607	5.0	1.0	408	2633	3323	3.7	0.83	0.76	0.72

* schedule A: capsule on day 1 and tablet on day 2

schedule B: tablet on day 1 and capsule on day 2

patient with total gastrectomy, excluded from further calculations

scale only. For C_{max} and AUC_{24h} the ratio's between the capsule and tablet formulation were calculated. Then, an analysis of variance (ANOVA) was performed to generate the appropriate estimates and mean square error to allow calculation of the 90% confidence intervals for these ratio's. A general linear model was used, including factors of gender, sequence, sequence with suubject, period, treatment and study site. Since there was no wash-out period between treatments, attention was paid to possible carry-over (CO) effects. If carry-over would have differed between treatments, then the estimate of the difference of the pharmacokinetic parameters between treatments would have been biased by $½(CO_{capsule} - CO_{tablet})$. However, the dominant half-life of R115777 is approximately 3 h, and hence no more than 10% carry-over in AUC values could be expected from day 1 to day 2. This potential bias was felt to be negligible. The values of the pharmacokinetic parameters of the patients who received doses of 400 mg, were standardized to a dose of 300 mg. This could be achieved by using a linear correction, since dose-linear pharmacokinetics in this range have been demonstrated for R115777 (12-14). For the bioavailability parameters C_{max} and AUC_{24h}, 90% classical confidence intervals of the ratio's tablet/capsule were calculated and bioequivalence was assumed when these intervals were within the critical range of 0.80-1.25 (15). Differences in T_{max} and $t_{½}$ between the treatments were statistically evaluated using a Wilcoxon test, preceded by a Friedman's test. All statistical calculations were performed using the software package Statistical Product and Service Solutions (version 6.1 for Windows, SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Twenty-four patients were included in the study, 11 males and 13 females, with a median age of 55 years (range 27-81). The tumor types of these patients included colon (n=7), lung (n=3), pancreas (n=1), cervix (n=1), stomach (n=3), liver (n=1), prostate (n=3) and renal (n=3) carcinomas. Two patients had adenocarcinoma of unknown primary. Thirteen patients received R115777 at a dose of 400 mg per day, the other eleven patients at a dose of 300 mg/day. This difference was due to the fact that the patients also participated in phase I dose escalating trials of R115777. One patient (patient number 6) with gastric carcinoma had had a total gastrectomy. The tablet/capsule ratio of the pharmacokinetic parameters in this patient were significantly lower than in all other patients, and he was excluded from the statistical analyses.

Pharmacokinetics

Pharmacokinetic parameters varied considerably between patients (Table I). The mean plasma concentration time curves for both formulations, standardized to a dose of 300 mg, are shown in figure 2. R115777 pre-dose levels on day 2 were all very low, on average 2.1% of the maximal plasma levels (range: 0-8 %), which was deemed insignificant. For the capsule formulation, T_{max} was 2.9 h (± 1.1), and for the tablet 2.5 h (± 1.1). This difference was not statistically significant. The half-life had a mean value of 2.9 h (± 1.1) for the capsule and 2.8 h (±0.9) for the tablet. As for the bioavailability parameters, mean ratio's, standard deviations and 90% confidence intervals are outlined in Table II. As can be seen, the ratio of each of the log-normalized parameters lies very close to 1, and the 90% confidence intervals fall within the 0.8-1.25 range.

Discussion

R115777 is a novel anticancer agent acting through inhibition of the farnesylation of Ras, Rho and other intracellular proteins. Because the antineoplastic activity of R115777 is a result of interference with signal transduction, which is a continuous process, the best effects of treatment might be expected from prolonged administration (16). Hence, a convenient dosage form should be available. R115777 can be administered via the oral route, with an estimated absolute bioavailability of 34 % ± 10, as determined in healthy volunteers (17). Results from the present study demonstrate that the tablet formulation meets the bioequivalence criteria when

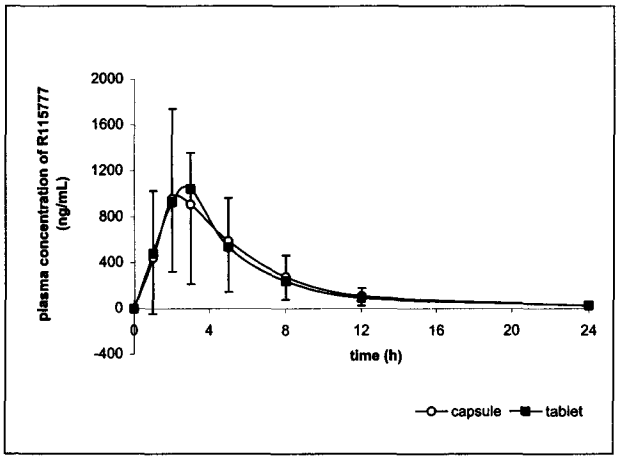

Fig. 2: Mean plasma concentration-time profiles of the capsule and tablet standardized to a dose of 300 mg R115777 qd

Table II: Tablet/capsule ratio's (mean and 90% confidence intervals) of the parameters C_{max}, and AUC_{24h} (n=23).

	ratio C_{max}	ratio AUC_{24h}
original scale		
mean	1.01	0.96
90% confidence interval	0.85-1.16	0.87-1.06
log-normalized		
mean	0.94	0.92
90 % confidence interval	0.81-1.09	0.83-1.03

compared to the capsule. R115777 has a short dominant half-life of less than 5 h, which allowed the trial design used here, lacking a wash-out period between treatments. This was demonstrated by the low trough levels of R115777 on day 2 and enabled us to include patients from the phase I trials also in the bioequivalence study without interrupting their treatment.

Interpatient variability in this study was considerable. From Table I, it can be seen that 4 patients (numbers 1, 5, 6 and 20) had tablet/capsule ratio's in AUC and C_{max} that deviated from the other patients. Patient number 6 had undergone a total gastrectomy, which may explain the observed difference. Patient number 1 was on ranitidine treatment (150 mg bid) and patients number 5 and 20 used omeprazole (40 and 20 mg qd, respectively) throughout the study. All of these patients had substantially lower values of C_{max}, AUC_{12h} and AUC_{24h} for the tablet than for the capsule. In vitro, the solubility of R115777 decreases with increasing pH (17), which may explain the observed effects. However, two other patients from our trial treated with cimetidine 400 mg qd (patient nr 4) and omeprazole 20 mg qd (patient nr 9) showed ratio's of these pharmacokinetic parameters that were in the normal range

(Table I). Thus, to establish the exact effect of agents interfering with the intragastric pH on the absorption of R115777, additional controlled studies are warranted. Phase III trials with R115777 and studies combining R115777 with classical cytotoxic agents are ongoing. As we have demonstrated, patients can be adequately treated with the tablet formulation of R115777. This will be of use especially when high dosage regimes are required.

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