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Clinical report

Phase I and pharmacologic study of the arotinoid Ro 40-8757 in combination with cisplatin and etoposide in patients with non-small cell lung cancer

Lia van Zuylen, Jan HM Schellens,[1] Swan H Goey, Linda C Pronk, Maureen M de Boer-Dennert, Walter J Loos, Jianguo Ma, Gerrit Stoter and Jaap Verweij

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital, 3008 AE Rotterdam, The Netherlands. [1]Present address: Department of Medical Oncology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.

This phase I study was performed to assess the feasibility of combining cisplatin/etoposide (VP-16) with the arotinoid Ro 40-8757 and to determine the dose-limiting toxicity (DLT) of Ro 40-8757 in this combination. Patients with non-small cell lung cancer were eligible. Treatment consisted of Ro 40-8757 p.o. day 1–21, cisplatin 100 mg/m^2 i.v. on day 2 and VP-16 100 mg/m^2 i.v. on day 2–4, repeated every 3 weeks. Eighteen patients were evaluable for toxicity and response. The doses of Ro 40-8757 ranged from 84 mg/m^2 once daily to 42 mg/m^2 thrice daily (tid). DLT consisting of delayed nausea/vomiting was reached at 42 mg/m^2 tid. Consequently, the maximum tolerated dose was set at one dose level below the DLT, i.e. 28 mg/m^2 tid. Skin toxicity occurred but was well manageable. Pharmacological analyses showed a small increase in the volume of distribution of cisplatin and VP-16 between the first and third course. However, no relationship with side effects was found. A response was achieved in 50% of patients. The combination of cisplatin/VP-16 with Ro 40-8757 appears to be feasible at a dose schedule of 28 mg/m^2 tid. The response rate was at the upper rate of what can be expected with cisplatin and VP-16. [© 1999 Lippincott Williams & Wilkins.]

Key words: Cancer, cisplatin, retinoids, vitamin A.

Introduction

Retinoids are a class of compounds structurally related to vitamin A. It has been known for many years that vitamin A has anticancer effects, both in prevention and therapy.[1] The mechanism of action involves induction of cell differentiation, inhibition of cell proliferation and induction of apoptosis (programmed cell death).[1,2] The toxicity profile of retinoids is similar to the hypervitaminosis A syndrome with leucocytosis/leucostasis, mucocutaneous toxicities, gastrointestinal toxicity (nausea/vomiting), musculoskeletal toxicity, ocular effects, CNS toxicity, hypercholesterolemia and hypertriglyceridemia.[2]

In the search for retinoic acid analogs that may be devoid of some of the side effects associated with the hypervitaminosis A syndrome, analogs with aromatic rings in the side chain, the so-called arotinoids, were developed.[3] Ro 40-8757 (mofarotene) is an arotinoid containing a morpholine structure in the polar end group.[3–5] *In vitro*, this compound had considerable anti-tumor activity against lung carcinoma, human breast cancer and colorectal cancer cell lines.[5] *In vivo*, Ro 40-8757 showed anti-tumor activity against established breast cancer in rats.[6] The exact mechanism of action is yet unrevealed.

A phase I study with Ro 40-8757 in patients with advanced solid tumors showed dose-dependent mucocutaneous toxicities.[3] Dose-limiting toxicity (DLT) was reached at 175 mg/m^2 single dose (sd) and consisted of WHO grade 3 vomiting. There was no myelosuppression.

As retinoids are not cytotoxic, they are unlikely to induce tumor regression in advanced solid tumors when used as single agent.[4] Therefore, combination with chemotherapeutic agents is considered of interest.

The prognosis of locally advanced or metastatic non-small cell lung cancer (NSCLC) is poor and one of today's best combinations consisting of cisplatin/etoposide (VP-16) yields response rates of only approximately 30%.

Sensitivity of NSCLC to antiproliferative effects of retinoids has been observed in experiments with

Correspondence to L van Zuylen, Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital, PO Box 5201, 3008 AE Rotterdam, The Netherlands.
Tel: (+31) 10 4391754; Fax: (+31) 10 4391003;
E-mail: l.van.zuylen@hetnet.nl

NSCLC cell lines, in which seven of nine lines showed growth inhibition after exposure to all-*trans* retinoic acid (ATRA).[8] However, clinical studies with ATRA or retinol mono-therapy in patients with advanced NSCLC have yielded disappointing responses of less than 10%.[2,9-12]

Only few studies have focused on the combination of retinoids with chemotherapy in NSCLC and the results were inconclusive.[9,10] Hence, we performed a phase I and pharmacologic study with the objective to develop a combination of cyclic cisplatin/VP-16 with chronic daily dosing of Ro 40-8757.

Materials and methods

Eligibility

Patients with histologically confirmed advanced or locally recurrent or metastatic NSCLC (stages IIIb and IV) for whom no therapies with greater potential benefit than cisplatin/VP-16 (and Ro 40-8757) existed were candidates for this study. Additional eligibility criteria were: age 18–75 years; Karnofsky performance status $\geqslant 70$; life expectancy of at least 3 months; no previous systemic anticancer therapy; no radiation therapy for at least 4 weeks; no previous treatment with Ro 40-8757 or with other retinoids; adequate bone marrow function (hemoglobin >6 mmol/l, white blood cell (WBC) count $>4\times10^9$/l and platelet count $>100\times10^9$/l), liver function (total bilirubin level <1.5 times upper limit of normal and AST <2.5 times upper limit of normal, or <5 times upper limit of normal if liver metastases were present), and renal function (serum creatinine <1.25 times upper limit of normal); serum calcium <2.6 mmol/l, cholesterol and triglycerides <1.25 times upper limit of normal. Written informed consent was required.

Pretreatment and follow-up studies

Before the start of treatment, the history of the patient was recorded, and physical examination, ophthalmic examination, laboratory studies, ECG, chest X-ray and an audiogram were performed. Computer tomographic scans were performed for tumor measurements. Laboratory studies included a complete blood cell count, differential WBC, electrolytes (sodium, potassium, chloride, calcium and inorganic phosphate), creatinine, urea, alkaline phosphatase, AST, ALT, bilirubin, protein, albumin, glucose, cholesterol, triglycerides, urinalysis and creatinine clearance. History, physical examination, including examination of the skin, toxicity scoring (according to the WHO grading system) and laboratory studies were repeated on days 8, 15, 23, 29, 44, 65, 86, 107, 128 and 169.

Ophthalmic (general exam, slit lamp exam, lens exam and ophthalmoscopy, all performed by an ophthalmologist) and oto-neurological tests were repeated on days 44, 86, 128 and 169. A final assessment was to be made after patients went off-study. Formal tumor measurements were performed at 6 weeks intervals until documentation of progressive disease (PD). Standard WHO response criteria were used.

ECG and chest X-ray were repeated as clinically indicated and when patients went off-study.

Drug administration

Cisplatin 100 mg/m^2 was administered every 3 weeks up to a maximum of eight courses on day 2 as a 4 h i.v. infusion diluted in 1 l of 0.9% saline. Before cisplatin administration, patients were hydrated with 1 l of 0.9% saline infused over 4 h and subsequent to cisplatin with 3 l of glucose/saline, supplemented with 3 g of potassium chloride and 2 g of magnesium sulfate, infused over 24 h.

VP-16 100 mg/m^2/day was administered every 3 weeks on days 2, 3 and 4 as a 60 min infusion reconstituted in 500 ml of 0.9% saline.

Anti-emetics included 5 mg i.v. tropisetron or 8 mg i.v. ondansetron, combined with 10 mg i.v. bolus dexamethasone, just prior to cisplatin infusion. For delayed nausea and vomiting, metoclopramide 20 mg thrice daily (tid) or domperidon 20 mg tid was given p.o. or per suppository.

If at the start of a treatment course (each 21 days) WBC were $>3\times10^9$/l and platelets $>100\times10^9$/l patients received full doses of cisplatin+VP-16. Treatment with cisplatin+VP-16 was delayed by 1 week (maximum 2 weeks) if on day 21 WBC were $<1.5\times10^9$/l or platelets were $<50\times10^9$/l. If WBC was between 1.5 and 3×10^9/l or platelets were between 50 and 100×10^9/l, the dose of VP-16 was reduced by 25%.

Ro 40-8757 dose

Ro 40-8757 (Hoffmann-La Roche, Strasbourg, France/Basle, Switzerland) was supplied as a 20% oral drinking solution preserved with sodium benzoate. It had to be protected from light. The drug was initially administered from day 1 on continuously as a single daily oral dose, each day at the same time and always within

30 min after ingestion of a glass of whole milk or a fat-containing meal, since absorption of retinoids is facilitated by ingestion of fats. While chemotherapy doses were fixed, Ro 40-8757 doses were escalated. Once the DLT of the single daily dose was reached, a 3 times daily regimen was investigated to determine if distribution of the total daily dose would enable a higher maximum tolerated dose (MTD) or a similar daily total dose with less toxicity. The rationale for splitting the total daily dose of arotinoid into three was based on observations in healthy volunteers, where it was observed that the concentration of the drug in the stomach rather than the plasma levels predicted the occurrence of nausea/vomiting. These data became available after the start of our phase I study.

In view of the long terminal half-life of Ro 40-8757 (7–10 days), splitting of the daily dose was not expected to affect steady-state levels, which were felt to be most important for potential efficacy. For reasons of patient compliance, the number of doses per day was limited to three. The Ro 40-8757 starting dose was set at 84 mg/m^2/day, which was one dose below the level associated with moderate (grade 2) toxicity in the single-agent phase I studies.[3] The dose was to be escalated according to a pre-established schedule and according to the toxicities observed at the previous dose level, after a minimum of three patients had been treated at a given dose level without DLT. Once side effects WHO $\geq$ grade 3 other than acute chemotherapy-related nausea/vomiting, constipation or hypertriglyceridemia were observed, at least three additional patients were entered at that dose level.

DLT was defined as WHO $\geq$ grade 3 organ toxicity noted in at least two out of six patients at a given dose level. For myelosuppression, DLT was defined as a granulocyte count less than 0.5×10^9/l with fever of 38.5°C or above requiring i.v. antibiotics.

For nausea/vomiting, constipation and hypertriglyceridemia, DLT was reached if symptomatic treatment of grade 2–3 toxicity did not improve these side-effects to at least grade 1.

The MTD was defined as the dose level below DLT. Six patients were to be studied at the MTD.

Pharmacokinetic studies

During treatment, blood samples were taken for assessment of the pharmacokinetics of Ro 40-8757, cisplatin and VP-16, and for measurement of cisplatin-DNA adducts.

For pharmacokinetics of Ro 40-8757, five samples (10 ml each) were taken on day 1 and 2 (before the first drug administration, and 1, 2, 4 and 8 h thereafter); then once daily on days 3, 4, 8, 15, 23, 29, 44, 65, 86, 107, 128 and 169, all before oral administration of Ro 40-8757. Following protein precipitation, Ro 40-8757 was extracted from plasma by liquid-liquid extraction. The concentration of Ro 40-8757 was determined by liquid chromatography with tandem mass spectrometry using atmospheric pressure ionization.

Drug monitoring of cisplatin and VP-16 and measurements of cisplatin-DNA adducts were performed during the first (days 2–4) and third (days 44–46) course of chemotherapy. On the first day one blood sample of 20 ml was taken just before the start of the infusion of VP-16 (time -1). In addition, samples were taken at 4, 4.5, 5, 6, 6.5, 20 and 22 h after the start of the infusion of cisplatin (time 0). One blood sample of 2.5 ml was taken at the end (C_{max}) of the 1 h infusion of VP-16.

Non-protein-bound cisplatin and the total DNA adduct levels of cisplatin in leucocytes were determined with flameless atomic absorption spectrometry according to the method of Reed, with modifications described by Ma *et al.*[13–15]

The concentrations of VP-16 were analyzed using high-performance liquid chromatography with UV detection at 210 nm. The system consisted of a Waters column packed with μBondapack Phenyl material (30 × 0.46 cm ID; 10 μm PS). The column was eluted with a mobile phase consisting of methanol:acetic acid:water (700:1:300, v/v/v) with a flow rate of 1.5 ml/min while the column was maintained at 60°C. Plasma samples of 1000 μl were extracted after addition of phenytoin, which was used as internal standard, with 5 ml of a mixture containing diethyl ether:dichloromethane:isobutyl alcohol (600:395:5, v/v/v) and vortex mixing for 5 min. After centrifugation the organic layer was collected and the extraction was repeated. The organic fractions were combined and evaporated to dryness at 50°C under vacuum. The residue was dissolved in a volume of 150 μl of mobile phase and 100 μl was injected into the HPLC system.

Pharmacokinetic analyses were carried out by using NONMEM (Nonlinear Mixed Effects Modeling; release 4.0, University of California San Francisco, CA). The plasma concentration-time data of cisplatin obtained during course 1 and 3 were fitted to a one-compartment linear model and those of VP-16 to a two-compartment linear model. A proportional error model was used. Estimates of clearance (CL) and volume of distribution (V_{ss}) were obtained using a Bayesian algorithm, which is one of the features of the program. Area under the plasma concentration-time curves (AUC) were calculated by dividing dose and clearance. Terminal half-lives ($t_{1/2}$) were calculated as $\ln 2/k$ (min), where k is the terminal elimination rate constant.

Statistical analysis

The paired two-sided Student's *t*-test was used to test for differences between the first and third course in pharmacokinetic results. The 95% confidence interval for the response was read from the table of the binomial distribution.

Results

Nineteen patients were entered into this study. Patient characteristics are listed in Table 1. One patient was considered not evaluable for toxicity and response. He started Ro 40-8757 at a dose of 42 mg/m^2 tid but had a seizure after the first dose of Ro 40-8757 due to previously unknown cerebral metastases.

Toxicity and compliance

The starting single daily dose of Ro 40-8757 of 84 mg/m^2 appeared to result in DLT. Subsequently, the schedule of Ro 40-8757 administration was changed from once a day to 3 times a day. The main toxicities per patient and per dose level are given in Table 2.

Table 1. Patient characteristics

No. of patients included	19
Male : female	11 : 8
Median age	53 (28–69)[a]
Karnofsky score	
100	1
90	16
70–80	2
Histology	
squamous cell carcinoma	4
adenocarcinoma	12
poorly differentiated	3
Stage of disease	
IIIb	9
IV	10
Prior therapy	
surgery	1
radiotherapy	3
chemotherapy	0
none	15

[a]Range in parentheses.

Table 2. Main toxicities (WHO grade)

Dose Ro 40-8757 (mg/m^2)	Patient no.	No. of courses	Skin toxicity			Neutropenia					Early N/V[a]				Late N/V[b]			
			1	2	3	0	1	2	3	4	0	1	2	3	0	1	2	3
84 sd	1	3	–	–	–	–	1	1	–	1	–	–	–	3	–	1	1	1
	2	6	–	1	–	–	–	1	2	3	–	–	4	2	1	–	2	3
	3	1	–	–	–	1	–	–	–	–	–	–	–	1	–	–	–	1
	4	8	1	1	–	4	1	–	2	1	7	1	–	–	6	2	–	–
	5	7	–	4	1	–	1	2	4	–	–	–	6	1	2	4	1	–
	6	3	–	2	–	1	2	–	–	–	–	2	1	–	–	3	–	–
Total	6	28	1	8	1	6	5	4	8	5	7	3	11	7	9	10	4	5
28 tid	7	8	–	–	–	1	2	1	3	1	3	2	3	–	3	1	4	–
	8	7	–	5	–	1	–	–	–	6	–	1	6	–	–	3	4	–
	9	2	–	–	–	–	–	–	2	–	–	–	1	1	1	–	–	1
	10	8	–	1	–	–	–	3	2	3	3	5	–	–	5	3	–	–
	11	6	–	–	–	–	–	–	3	3	–	2	4	–	2	1	3	–
	12	8	–	–	–	–	–	–	3	5	5	–	3	–	3	1	3	1
	18	2	–	–	–	–	–	–	2	–	1	1	–	–	2	–	–	–
Total	7	41	–	6	–	2	2	4	15	18	12	11	17	1	16	9	14	2
42 tid	13	5	–	–	3	–	–	–	1	4	1	1	3	–	–	–	5	–
	14	8	–	–	–	–	2	–	1	5	2	1	3	2	–	4	2	2
	15	6	–	1	–	1	1	–	3	1	–	–	6	–	–	–	5	1
	16	4	–	–	–	–	3	–	1	–	–	1	–	3	1	–	3	–
	17	5	–	–	–	1	–	–	1	3	–	–	5	–	1	–	4	–
Total	5	28	–	1	3	2	6	–	7	13	3	3	17	5	2	4	19	3

[a]Nausea/vomiting occurring during the first 5 days after chemotherapy.
[b]Nausea/vomiting during day 6–21.

Due to delayed nausea/vomiting, which lasted until day 9–14 and did not respond to oral metoclopramide or domperidon, three of six patients at the dose of 84 mg/m^2 sd had to end Ro 40-8757 treatment early. So, this dose qualified for DLT. One of these patients also experienced conjunctivitis grade 3. Skin toxicity occurred in 10/28 courses (35%) and grade 3 occurred in one patient in course 2, but with the use of skin lotion this side effect could be alleviated and termination of treatment could be avoided. Dose reduction of VP-16 according to protocol was necessary in three patients. Dose reduction of cisplatin was necessary in one patient after course 5 because of nephrotoxicity grade 1. One patient had to end the treatment after the seventh course due to nephrotoxicity grade 1 and neurotoxicity grade 2. Only one patient received the scheduled eight courses. Drop outs were mainly due to chemotherapy and Ro 40-8757 related side effects.

At 28 mg/m^2 tid, delayed nausea/vomiting was manageable. Two patients experienced grade 3 delayed nausea and vomiting, but only for 1 or 2 days and not leading to interruption of intake of Ro 40-8757. Skin toxicity occurred in six of 39 courses (15%) and again was found manageable. Two patients were admitted because of neutropenic fever, respectively, after the first and third course. After reducing the VP-16 dose they received, respectively, seven and eight courses. Because these neutropenic fevers were clearly chemotherapy related, and since these two events could be explained in the light of the normal frequency of neutropenic fever with this schedule and these doses of chemotherapy, it was decided not to count them as DLT. According to the protocol, the VP-16 dose had to be reduced in one other patient because of WBC 2.5×10^9/l on day 21. After the first course, one patient experienced a mental depression. He was treated with fluoxetine and was able to receive all eight scheduled courses.

At 42 mg/m^2 tid five patients were evaluable. Because of vomiting directly after intake of Ro 40-8757, one patient used a 50% dose of Ro 40-8757 from course 2 on. Persisting vomiting in this patient was the reason to end the treatment after six courses. Three out of these five patients developed grade 3 delayed nausea/vomiting that occurred between day 8 and 16, and leading to interruption of intake of Ro 40-8757 for a maximum of 1 day. Therefore, DLT was reached at this dose level. One patient developed skin toxicity grade 3. One patient became depressed after course 4 and was started on fluoxetine. One patient was admitted with fever on day 21 after the first course. Although this did not coincide with neutropenia, the second course was delayed by 1 week. Due to nephrotoxicity grade 1, dose reduction of cisplatin was necessary in one patient after the third course and another one had to stop cisplatin after the sixth course. In view of the companies decision to stop further development of the drug, only one further patient could be entered at the level that would be recommended as for further phase II/III studies, i.e. 28 mg/m^2. This patient received two courses, but had to withdraw further treatment because of hearing loss confirmed by audiometry. There was no toxicity due to Ro 40-8757.

Symptoms of the hypervitaminosis A syndrome were seen in 13 patients. There was no apparent dose dependency. Dryness of skin or mucous membranes was most obvious (70% of patients), but also eye and orbital abnormalities were found (40% of patients). The latter included chalazion, blepharitis, conjunctivitis, edema of the papil and small intraretinal bleeding. All of these side effects were manageable and never a reason to stop the treatment.

Partial response of the tumor was noted in 50% (95% CI 0.25–0.75) of the entered patients. When the response rate was distinguished for stage IIIB and stage IV NSCLC, it was 66 and 33%, respectively.

Pharmacokinetics

The pharmacokinetics of cisplatin could well be fitted to a one-compartment and those of VP-16 to a two-compartment linear model. The main pharmacokinetic results of cisplatin, VP-16 and cisplatin-DNA adducts are shown in Table 3.

Comparison of the data obtained during course 1 and 3 revealed that the CL and AUC values were not significantly different for cisplatin and VP-16. In contrast, small but statistically significant differences were found in the V_{ss} (both compounds) and terminal $t_{1/2}$ (VP-16). The terminal $t_{1/2}$ of cisplatin was also longer during course 3, which is in line with the higher V_{ss}, but this did not reach statistical significance. The significantly lower C_{max} of VP-16 during the third course was compatible with the higher V_{ss}. There was no indication that the dose of Ro 40-8757 influenced the pharmacokinetic results of cisplatin and VP-16 nor the formation of cisplatin-DNA adducts.

The maximum concentration of Ro 40-8757 measured in the six patients who were using the drug once a day was reached between 2 and 4 h after intake for all patients except one (Figure 1). For the patients who used the arotinoid 3 times a day, the maximal concentration was reached 8 h after the first intake.

Table 3. Main pharmacokinetic parameters of cisplatin and VP-16 obtained during the first and third course of administration

	C_{max}[a]		CL (l/h)		AUC (μg·h/ml)		V_{ss} (l)		Terminal $t_{1/2}$ (h)	
Cisplatin										
course	1	3	1	3	1	3	1	3	1	3
mean	0.89	0.97	50.9	46.7	2.74	2.56	43.6	52.5[b]	0.60	0.68
SD	0.12	0.25	4.9	5.0	0.29	0.56	5.4	7.5	0.12	0.18
range	0.61–1.09	0.63–1.44	46.1–63.3	43.5–61.9	2.63–3.58	2.00–4.03	43.7–62.5	48.4–80.1	0.48–0.93	0.54–1.27
N	15	13	15	13	15	13	15	13	15	13
VP-16										
course	1	3	1	3	1	3	1	3	1	3
mean	19.66	15.71[b]	5.37	4.92	29.8	31.8	29.8	32.4[b]	3.62	4.30[b]
SD	2.49	3.24	0.94	0.70	4.1	3.9	1.1	2.6	0.55	0.53
range	15.66–23.02	11.22–19.88	4.02–7.93	4.72–7.08	20.8–40.3	27.6–42.3	28.8–33.1	29.6–40.2	2.66–5.33	3.15–5.78
N	14	10	14	10	14	10	14	10	14	10
Cisplatin–DNA adducts										
course	1	3								
mean	1.12	1.51[b]								
SD	0.51	0.50								
range	0.53–2.05	1.02–2.51								
N	7	7								

[a] C_{max} for cisplatin and VP-16 in μg/ml; C_{max} for cisplatin–DNA adducts in pg Pt/μg DNA.
[b] $p<0.05$.

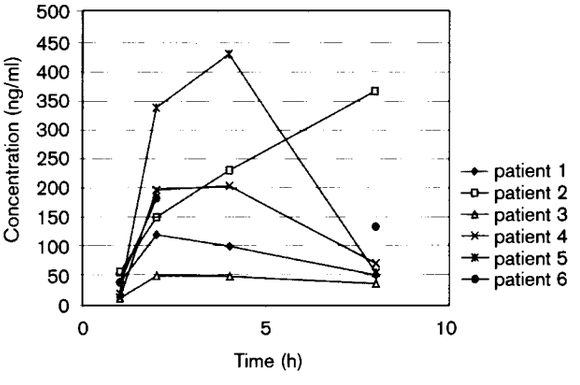

Figure 1. Pharmacokinetics of Ro 40-8757 84 mg/m^2 sd, day 1.

Discussion

The relationship between vitamin A and cancer was first noted in the 1920s, when experimentally induced vitamin A deficiency was shown to induce preneoplastic lesions and ultimately neoplasms. In the 1970s it was found that vitamin A could also have a therapeutic effect on cancer. This antitumor effect was not only associated with vitamin A (retinol) but also with the metabolite vitamin A acid (ATRA).[1] The retinoids are a class of compounds structurally related to vitamin A.[1] Retinoids are involved in regulation of gene expression and in numerous immunomodulatory effects.[10] Most retinoids are known to bind to a group of nuclear receptors which belong to the same superfamily as those which mediate steroid hormone activities.[1,2] Retinoid binding proteins (previously mistaken for receptors) are responsible for the intracellular transport of retinoids and the regulation of retinoid concentration.[1] The anti-tumor activity observed *in vitro* is mainly the result of cell differentiation and inhibition of cell proliferation.[1,2] The third generation of retinoids, the arotinoids, are analogs with aromatic rings in the side chain.[3,16] Ro 40-8757 is an arotinoid containing a morpholine structure in the polar end group.[5,6] After oral administration there was little first-pass metabolism and the bioavailability in man was approximately 60%. Little of the drug was eliminated in bile or urine (less than 1%), suggesting predominant metabolic clearance. Tissue penetration was high with a half-life of approximately 20 days following multiple administrations. This was felt to be consistent with some accumulation in body fat.[3]

In vitro and *in vivo*, Ro 40-8757 showed anti-tumor activity against different cell lines and against established breast cancer in rats. The mechanism of action of Ro 40-8757 is unrevealed. In well-established receptor-binding assays, it has been shown that Ro 40-8757 does not bind to any of the five well-characterized retinoic acid receptors. It has been suggested that part of the anti-tumor effect may be through impairment of mitochondrial function.[3,5]

Retinoids were reported to exert anti-angiogenic effects, inhibit cell growth, induce cell differentiation and induce apoptosis. Induction of apoptosis may represent an important mechanism by which cytostatic agents as well as retinoids inhibit tumor growth.[17] Recently, Aebi *et al.* reported a synergistic effect of ATRA and cisplatin in some human cell lines. ATRA seems to enhance the potency of cisplatin by increasing the susceptibility of the cells to undergo apoptosis through down-regulation of Bcl-2, a protein which inhibits apoptosis.[18]

The prognosis of inoperable NSCLC is poor. Recent meta-analyses showed that cisplatin-containing chemotherapy offers a limited survival benefit.[19,20] In Europe the combination of cisplatin with VP-16 is widely used. However, this combination yields response rates of only approximately 30% with a median survival of 6–9 months.[7] New agents, preferably with other mechanisms of action, are therefore urgently needed. Retinoids may be one of the options, especially in view of the possible synergism in the combination with cisplatin. Treatment with single-agent ATRA in patients with metastatic NSCLC and other solid tumors showed disappointing response rates in two recent phaseI/II studies.[11,12] The results of phase II studies in metastatic NSCLC involving etretinate and isotretinoin plus different regimens of cytotoxic agents were inconclusive.[9,10] Our phase I study, combining the arotinoid Ro 40-8757 with cisplatin/VP-16 for the treatment of advanced metastatic NSCLC, showed an overall response rate of 50 and 66% for stage IIIB NSCLC, and thus synergy of this particular combination remains a possibility that requires further study.

Side effects characteristic of hypervitaminosis A syndrome are common with retinoid treatment in cancer patients. Frequent, but almost always manageable are the mucocutaneous toxicities (dryness of mucosal tissues, erythema and desquamation of skin, cheilitis). Ocular effects (particularly dryness of the eyes and blepharo-conjunctivitis) are seen and also corneal erosions have been reported. A dose-response relationship is seen for CNS toxicity (headache, psychologic changes) and for gastro-intestinal toxicity (nausea/vomiting). Laboratory abnormalities include elevated serum liver enzymes as a manifestation of reversible hepatotoxicity, as well as hypertriglyceridemia and hypercholesterolemia.[2,9]

In the phase I study we report here, DLT consisted of gastrointestinal toxicity (delayed nausea/vomiting) at the dose of 84 mg/m^2 sd. Observations in healthy volunteers meanwhile showed that stomach, rather than plasma concentrations of Ro 40-8757 predicted nausea and vomiting. Consequently, we divided the daily dose of Ro 40-8757 into 28 mg/m^2 tid. This was much better tolerated at the dose level with the same total dose of 84 mg/m^2/day. DLT, consisting again of gastrointestinal toxicity, was now reached at 42 mg/m^2 tid. The other side effects observed confirm previous data from the literature.[2] Most patients experienced dryness of skin or mucous membranes, although in various degrees. Only a few experienced moist desquamation, but this was always manageable with local treatment. Almost half the patients complained about visual disturbances, although this was never a reason to stop Ro 40-8757. Two patients consulted a psychologist/psychiatrist because of psychological complaints. It was not clear if these were related to Ro 40-8757 or to the illness. No significant change was found in liver function tests or plasma triglyceride concentration.

We found no relationship between the pharmacokinetics of cisplatin and VP-16 and the observed side effects. Small but statistically significant differences were found in the V_{ss} of cisplatin and VP-16, where the V_{ss} data of course 3 were higher than of course 1. The CL values illustrate that this parameter was unchanged when comparing the first and third course. The cisplatin–DNA adduct levels in WBC during the third course were higher than during the first course. This is in line with previous observations and indicates accumulation of adducts in WBC upon repeated treatment.[21] The magnitude of the accumulation is of the order as previously described. Therefore, a contributon of the co-administered compounds VP-16 and Ro 40-8757 appears to be unlikely.

Based on the response rates we observed and based on the recently published study from Aebi *et al.*,[18] a synergistic effect with cisplatin/VP-16 might be postulated, but obviously further studies including phase III trials would be needed to confirm this. In case of a phase II/III study with the combination of Ro 40-8757 and cisplatin/VP-16, 3-weekly scheme, the recommended dose of Ro 40-8757 will be 28 mg/m^2 tid.

Conclusion

Treatment of patients with advanced or metastatic NSCLC with the combination of the arotinoid Ro 40-

8757 and the two cytotoxic agents cisplatin and etoposide was found to be feasible. The MTD of Ro 40-8757 in the combination was 28 mg/m^2 tid, day 1-21. DLT was noted at the dose level of 42 mg/m^2 and consisted of delayed nausea/vomiting. Mucocutaneous toxicities and ocular effects, as seen in hypervitaminosis A syndrome, occurred but were always well manageable and never a reason to stop treatment. No relationship was found between the side effects and the pharmacokinetics of cisplatin and VP-16. The response rate of 50% was at the upper rate of what can be expected with cisplatin/VP-16 for the treatment of advanced or metastatic NSCLC and a possible synergistic effect might thus be postulated. Based on recent results, the enhancement of the potency of cisplatin by retinoids could possibly be explained through down-regulation of Bcl-2, a protein which inhibits apoptosis. Further studies including phase III trials would be needed to confirm the possible synergism.

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