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A phase 1 and pharmacokinetic study using the aromatic retinoic acid analogue dichloroetretinate (Ro 12-7554)

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Summary. A phase I study was carried out with the new aromatic retinoic acid analogue DCE, all-*trans*-9-(2,6-dichloro-4-methoxy-*m*-tolyl)-3,7-dimethyl-2,4,6,8-nonatetraenacetylester. Data from preclinical studies show that DCE has a promising anti-tumor effect. Data from others investigators show that when DCE was given to patients in daily doses, the dose-limiting toxicity. This toxicity was comprising considerable muco-cutaneous toxicity, occurred at 40 mg/day. To avoid this dose-limiting toxicity, a weekly oral treatment schedule was tested for toxicity in this study. The starting dose was 40 mg/m^2 body surface, and a modified Fibonacci scheme was used for the dose escalations. A total of 20 patients entered this study, and all were evaluable for toxicity. The highest dose was 300 mg/m^2. In three patients, completely reversible WHO grade 1 liver toxicity was observed. In contrast to daily doses, a once-a-week schedule produced no muco-cutaneous toxicity. Pharmacokinetic measurements showed that absorption was highly unpredictable and did not increase with dose increments. Given the results of the pharmacokinetic determinations, we concluded that escalating the DCE dose would not lead to a recommendable dose for further phase II studies, and the study was subsequently discontinued.

Introduction

Animal studies [18, 24] have revealed a promising anti-tumor effect for retinoids. In vitro studies with tumor-cell lines derived from humans [25] suggest a possible anti-tumor effect. However, the mechanism underlying these anti-tumor effects differ from those of cytostatic drugs that are used in clinical practice. The activity of retinoids is more like that of steroid hormones. The observed effects of retinoids, such as terminal differentiation of malignant cells [2, 26], restoration of contact inhibition [10], and activation of subsets of lymphocytes [19], could enhance the inhibition of tumor growth. Whether these effects occur in humans is unknown, but recent work suggests that at least the effect on terminal differentiation of tumor cells can be observed in vivo [11].

In the clinical studies thus far published, mixed results have been reported in the treatment of malignant tumors. Some positive results have been noted in the treatment of frequently relapsing basal-cell carcinomas [28], superficial bladder tumors [21], myelodysplastic syndromes [14], and in some patients with promyelocytic leukemia [11]. The observed absence of beneficial effects might be due to suboptimal dosing schedules. When retinoids are given to humans on a daily dose schedule, extensive scaling of the skin, itching, cheilitis, alopecia and skin fragility occur after a few weeks of treatment. This toxicity is usually dose-limiting and may occur ar doses that vary considerably between patients. Liver toxicity [27] increases in blood lipids [1, 24], bone abnormalities [12], and muscle damage [17] have also been observed.

In an attempt to obviate skin toxicity, many different analogues have been synthesized. One of the analogues of retinoic acid, etretinate, is now widely used in dermatology for a variety of skin diseases [9]. A new analogue, all-*trans*-9-(2,6-dichloro-4-methoxy-*m*-tolyl)-3,7-dimethyl-2,4,6,8-nonatetraenacetylester (dichloroetretinate, DCE, Ro 12-7554; see Fig. 1), synthesized by Hoffmann-La-Roche (Basle, Switzerland), showed a better therapeutic index in the animal model than did retinoic acid. DCE has a better therapeutic index than either all-*trans* retinoic acid or etretinate in the B-16 melanoma model in the mouse, the Crocker chondro-sarcoma model in the rat, and the DMBA-induced skin papilloma in the mouse (G. Stüttgen, unpublished data). An interesting feature of DCE is the occurrence of a positive dose-response relationship with regard to its anti-tumor effect.

Another approach to reduce the toxicity associated with retinoid administration, besides changes in the molecular structure of these agents, might be the use of higher doses and longer dose intervals. A recently published study [4] showed a better tolerance for weekly 13-*cis* retinoic acid than was achieved with a daily dose. When DCE was used in a daily dose (phase I) study (G. Stüttgen, unpublished data), dose-limiting skin toxicity occurred after to weeks of treatment at doses ranging from 20 to 40 mg. The present article describes the results of a study using once-a-week administration of DCE in cancer patients to obtain a starting dose for further phase II studies.

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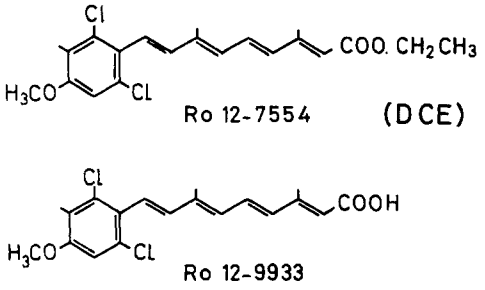

Fig. 1. Molecular structure of DCE and Ro 12-9933

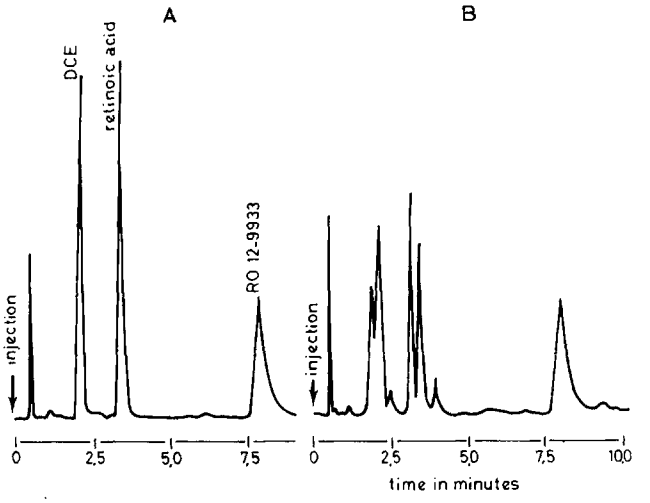

Fig. 2. **A** Chromatogram of a 1-ml plasma extract, spiked with 100 µg/l DCE (2.29), 100 mg/l Ro 12-9933 (8.43 min) and 50 mg/l retinoic acid (3.75). **B** Chromatogram of decomposed standards

Patients and methods

Patients. The patient protocol of this phase I study was approved by the Utrecht University Hospital Ethical Committee. A total of 20 patients entered the study after giving their witnessed, informed consent; their characteristics are summarized in Table 1. Patients were eligible to participate if progression of a histologically proven tumor for

Table 1. Summary of patient characteristics

Patient number	Sex	Age (years)	Histological diagnosis	Previous therapy	Weekly dose of DCE (mg/m^2)	Number of DCE doses
1	m	31	Soft-tissue sarcoma	surg, X-ray, ADR, MTX, MMC	40	4
2	f	42	Thyroid cancer	surg, X-ray, MMC, ADR, CDDP, VP 16	40	16
3	f	70	Adenoid cystic cancer	surg, X-ray, ADR, 5-FU, CDDP	40	8
4	f	60	Ovarian cancer Breast cancer	surg, X-ray, HMM, ADR, CDDP, CTX, 5-FU, MTX, MMC	40	8
5	m	64	Prostatic cancer	surg, X-ray, CTX, 5-FU	40	3
6	m	50	Head and neck cancer	surg, X-ray, MTX, dThD, CDDP, BLM, 5-FU	40	3
7	m	49	Lung cancer	None	50	11
8	f	57	Breast cancer	surg, X-ray, 5-FU, MMC, ADR, CTX, MTX, VCR, horm	50	11
9	m	46	Colon cancer	surg	50	8
10	m	54	Colon cancer	surg	50	8
11	m	78	Soft-tissue sarcoma	surg, X-ray, ADR	75	8
12	m	73	Anus cancer	surg, X-ray	75	8
13	m	54	Colon cancer	surg	75	19
14	m	33	Soft-tissue sarcoma	surg, X-ray, ADR, 4-EPI ADR	135	5
15	m	40	Acup	None	135	6
16	m	73	Mesothelioma	None	200	6
17	m	67	Lung cancer	X-ray	200	8
18	m	65	Lung cancer	None	200	15
19	f	41	Breast cancer	surg, X-ray, CTX, MTX, MMC, 5-FU, horm, VCR, ADR	300	11
20	m	72	Lung cancer	X-ray	300	13

surg, surgery; X-ray, radiotherapy; horm, hormonal therapy; ADR, doxorubicin; MTX, methotrexate; MMC, mitomycin C; CDDP, cisplatin; CTX, cyclophosphamide; VP 16, etoposide; 5-FU, fluorouracil; HMM, hexamethylmelamine; dThD, thymidine; BLM, bleomycin; VCR, vincristine; 4-EPI ADR, 4-epirubicin; Acup, adenocarcinoma of unknown primary site

Table 2. Summary of pharmacokinetic data

Patient	8.1	8.2	8.3	9	11	12	13	14	15	19
Dose:										
mg	90	80	80	100	140	140	140	220	220	400
mg/m^{2}BSA	56	50	50	50	75	75	75	135	135	300
AUC_0^{∞}($\mu g \cdot h \cdot l^{-1}$):										
DCE	1,420	915	561	278	2,671	1,148	1,614	2,089	20	1,922
Ro 12-9933	668	1,310	447	102	1,733	708	348	3,804	208	2,789
C_{max}($\mu g \cdot l^{-1}$):										
DCE	140	76	103	44	310	195	140	260	14	400
Ro 12-9933	40	90	32	10	92	45	45	300	35	250
T_{max}(h):										
DCE	6	4.5	4.5	8	6	4	6	6.5	5.5	4.5
Ro 12-9933	3.3	4.5	3.4	9	8	4.6	6.5	6	5	4.0
Ratio AUC_0^{∞}Ro 12-9933/AUC_0^{∞}DCE:										
	0.48	1.43	0.80	0.37	0.65	0.62	0.22	1.82	10	1.45
$\lambda_z(h^{-1})$:										
DCE	0.1383	0.0352	0.1777	ND	0.0507	0.0788	0.0159	0.0400	ND	0.3647
Ro 12-9933	0.0606	0.0260	0.0797	ND	0.0202	0.0552	0.3035	0.0245	0.3080	0.1293
Cl/F ($l \cdot h^{-1}$):										
DCE	63.4	87.6	142.6	359.7	52.4	122	86.7	1053	ND	208.1

Patient 8.2 represents the second course of DCE in patient 8; 8.3 represents the last course in patient no 8; 80 mg DCE dissolved in 70/30 ethanol/water was delivered ND, not done; BSA, body surface area

which no effective treatment was available had been diagnosed. The clinical performance status was WHO grade 2 or better, and all patients had an estimated life expectancy of >2 months [20]. Patients were considered evaluable for toxicity after three doses of DCE.

The WHO scoring system was used to assess toxicity [20]; dose-limiting toxicity was considered to be WHO grade 2 or more. The maximum tolerated dose was reached when at least two patients experienced grade 2 toxicity at a given dose level [20]. After 8 weeks of treatment, tumor response was determined according to standard WHO criteria [16, 20]. When progression was detected or a patient refused further treatment, optimal palliative care was provided. Patients were seen at least once a week, generally on an out-patient basis. At weekly intervals an extensive hematological and biochemical profile was determined.

Drug treatment. DCE was provided in 10- and 20-mg gelatin capsules that were swallowed with 150 ml water before breakfast after an overnight fast. The drug was supplied by Hoffmann-LaRoche B. V. (Mijdrecht, The Netherlands). The starting dose was 40 mg/m^2, which was shown to be tolerable on the previously mentioned daily dose schedule. The doses were increased according to the modified Fibonacci scheme [3]. The individual doses and the quantity of doses given are summarized in Table 1. Patient 8 was also sampled in week 2 (*pat. 8.2*). In a study of relative biovailability, patient 8 (Table 2, *pat. 8.3*) received one dose of 80 mg DCE dissolved in a 100-ml mixture of water/ethanol (30/70) after the trial as a reference.

Plasma level determinations. Plasma concentrations of DCE and the active metabolite Ro 12-9933 were determined in eight patients by venous sampling via a heparinized catheter at different time points (for exact time points see Fig. 2). All sampling was done in the absence of fluorescent lights in a shaded room, and blood was collected in heparinized plastic tubes (Sarsted, Eindhoven, The Netherlands). After the samples had been drawn, the tubes were immediately wrapped in aluminum foil (to prevent photo-isomerisation) and subsequently refrigerated at 4° C. As soon as possible, the samples were centrifuged and plasma was removed in a specially darkened laboratory room with only filtered fluorescent light illumination (Philips 40 W, color 17; wrapped with Toverli Lee Filter 107). Plasma was quickly frozen and stored in a light-proof container at $-30°$ C.

Determination of DCE and Ro-9933. Samples were analyzed by adapting the HPLC method described by Hänni et al. [15] for the determination of etretinate [23]. Waters HPLC equipment was used (Waters; Etten-Leur, The Netherlands) to carry out an isocratic elution procedure with hexane/tetra-hydrofuran/glacial acetic acid (1,000/3.5/3.0) as a mobile phase at a flow rate of 1.5 ml/min. As a stationary phase, a Partisil (5 µm silica, spherical) column (15 cm; inside diameter, 3 mm) was used; as a precolumn, a 7.5-cm pelllicular silica column (inside diameter, 2.1 mm) was used. Both columns were obtained from Chrompack B. V. (Middelburg, The Netherlands). UV detection was done at 360 nm.

Samples were prepared in brown glasswork. To 1 ml plasma we added 50 ng retinoic acid in methylacetate (as

an internal standard) and 800 µl formic acid. After this was vortexed for 5 s, 10 ml hexane was added and the mixture was shaken for 10 min and centifuged for 10 min at 2,000 *g*. The hexane layer was dried by nitrogen at 25° C and the residue was dissolved in 100 µl HPLC eluate.

Samples of 75 µl were injected onto the column. Linearity was proven for DCE and RO 12-9933 between 0 and 400 mg/l plasma. The correlation was >0.99, and relative recovery was 94%±3% on spiked plasma samples. To control for variability during the whole procedure, samples exposed to daylight (see Fig. 2) were analyzed prior to and during every run to detect photo-isomerisation (and thus incorrect sample preparation) and check the separation capacity of the column. The lowest quantifiable amount of both substances was 5 ng/ml plasma.

Pharmacokinetic parameters. Plasma concentration vs time data of both DCE and Ro 12-9933 formed the basis for calculating the parameters indicated in Table 2 using standard formulas [13, 15]. The terminal rate constant λ_z, was calculated by means of (log concentration vs time) regression analysis on the terminal portion of the plasma concentration-time profile. The areas under the plasma concentration-time curves (AUCs) were calculated using the linear trapezoidal rule up to the final measurable plasma concentration ($C_{p,\,last}$) and then extrapolated to infinity using the equation:

$$AUC_0^\infty = AUC_{C_0}^{Cp,\,last} + \left(\frac{C_{p,last}}{\lambda_z}\right) .$$

C_{max} and T_{max} were obtained by graphic inspection, and Cl/F was obtained from the relationship:

$$Cl = \left(\frac{FxD}{AUC_0^\infty}\right) ,$$

where Cl is the clearance in $l \times h^{-1}$, D is the dose in µg, AUC_0^∞ is the area under the curve in $\mu g \times h \times l^{-1}$ of the parent compound and the metabolite RO 12-9933, and F is the bioavailability (fraction of dose; no dimension). An Apple II computer was used to do the calculations.

Results

In all 20 patients were entered in this study, and all were evaluable for toxicity; only 13 were evaluable for tumor response. The dose was not increased above 300 mg/m^2 once every week because pharmacokinetic observations in eight patients showed that dose increases did not lead to predictable increases in absorption. A total of 181 doses of DCE were given (3–19 doses/patient). Toxicity of skin and oral mucosa was minimal and consisted of minor complaints of dry lips. These complaints occurred only in a minority of the patients and did not necessitate changes in therapy. Liver toxicity was noted in three patients (patients 16, 18 and 20 in Table 1) and consisted of WHO grade 1 rises in transaminases and alkaline phosphatase. All changes disappeared after the cessation of therapy.

One patient (number 18) experienced transient grade 1 vomiting 1 day after taking the drug. In two patients, minor (WHO grade 0) serum creatinine rises were noted. Patient 10 combined DCE with 1.2 g glafenine (an analgesic with known nephrotoxicity at high doses) [8]. His serum creatinine levels rose from 50 to 120 µmol/l. After

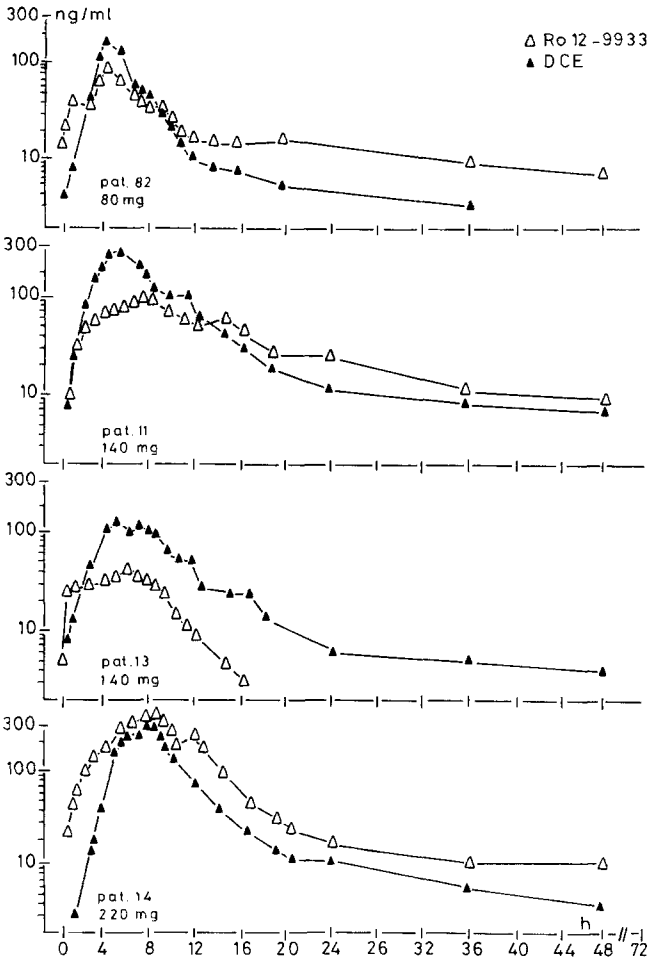

Fig. 3. Plasma levels of DCE and Ro 12-9933 in patients (*pat.*) 82, 11, 13 and 14

discontinuation of both drugs, his serum creatinine returned to normal after 1 week, but a few days after the re-challenge with DCE the patient died suddenly at home, most probably as a consequence of a myocardial infarct (no autopsy was done). Patient 18 had a slight increase in serum creatinine levels, from 109 to 15 µmol/l, when he combined chlorthalidon with DCE. After cessation of DCE, his serum creatinine returned to 112 µmol/l. Although no objective responses were seen, in seven patients states of stable disease were recorded for periods of up to 19 weeks.

Individual plasma concentration-time curves are shown in Figs. 3 and 4. The calculated parameters are given in Table 2. A plot of the dose in mg/m^2 body surface area (BSA) in relation to AUC_0^∞ for both DCE and its metabolite Ro 12-9933 shows only weak correlations of $r = 0.44$ and $r = 0{,}67$, respectively (Fig. 5), as does a plot of $C_{max\ DCE}$ ($r = 0.49$; Fig. 5). Patient 15 was omitted from all calculations, as he probably suffered from some sort of malabsorption. Patient 8 was monitored for two consecutive courses (numbers 8.1 and 8.2). The "reference dose" was given after the end of her study period. In this patient, major variations in AUC_0^∞ and C_{max} were noted, although the delivered doses were almost identical. These variations can be explained by variable absorption.

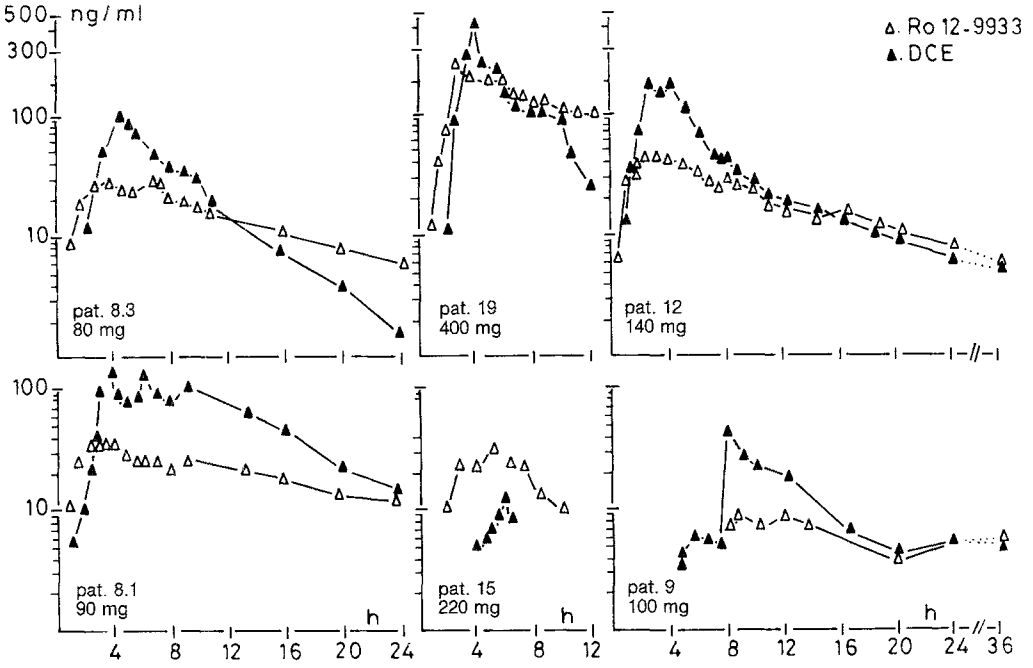

Fig. 4. Plasma levels of DCE and Ro 12-9933 in patients (*pat.*) 8.3, 19, 12, 81, 15 and 9

Although the number of patients in the groups given 50 and 75 mg/m^2 DCE are too small for statistical evaluation, there seems to be a difference in the mean AUC_0^∞ and C_{max} values between both groups. In contrast, however, large variations in these parameters can be noted within one group. In patients 8.2, 14, and 19, the ratio AUC_0^∞ Ro 12-9933/AUC_0^∞ DCE exceeds unity, meaning that in these patients metabolite clearance must be lower than clearance of the parent compound [23]. The Cl/F values range from 52.4 to 1053 l/h, with no relationship to dose, indicating a large variability in F. By definition, F × dose = clearance × are [23]. Increasing the dose produces a proportional increase in the plasma concentation at all times, unless the absorption half-life or the bioavailability have been altered. Hence, the time for the peak remains unchanged but its magnitude increases proportionally with the dose. In Fig. 5, T_{max} was plotted vs dose and no indication of rigorous changes in absorption half-life was detected.

Given the limitations of a phase I study (testing for toxicity in a limited number of observations in severely ill patients), a drug formulation with a sufficiently reliable bioavailability is essential for its evaluation. From the results discussed above, we concluded that the currently studied formulation of DCE is not suitable for reliable observations and that further dose escalations and pharmacokinetic investigations are not justified. As an intravenous formulation was not available, the DCE study was discontinued.

Discussion

The vast amount of data from in vitro and animal studies would suggest a more prominent role for retinoid treatment than has been observed in subsequent clinical studies. The positive effect of DCE in animal tumor models suggests that this retinoid might be particularly suited for use in oncology. Because most retinoids are prescribed on a daily dose schedule in analogy to treatment with steroids, there is a fair chance that current disappointment in clinical results could at least be partly, explained by the suboptimal dose schedules used.

Skin and mucosal toxicity of most retinoids are quite unpleasant and often necessitate a reduction in the dose after a few weeks of treatment. In the present study, skin

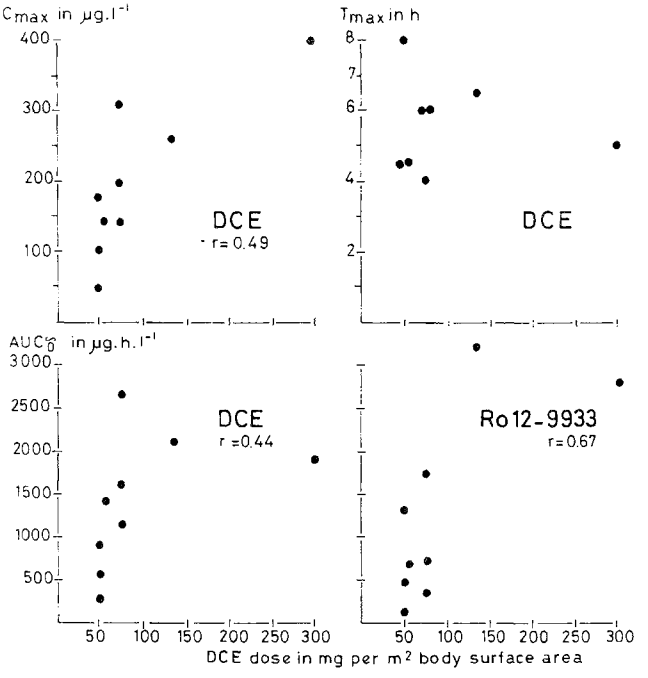

Fig. 5. Plots of C_{max} vs DCE dose, T_{max} vs DCE dose, AUC_0^∞ DCE vs DCE dose, and AUC_0^∞ Ro 12-9933 vs DCE dose

and mucosal toxicity were not dose-limiting; only minor and reversible liver-function disturbances were noted. The liver toxicity that was observed in this study is not uncommon for retinoids. Daenen et al. [7] have suggested that the esterified form of various retinoic acid analogues accumulates in certain liver cells. However, this hypothesis is doubtful, given the results presented by Rollman and Vahlquist [22] for the aromatic retinoic acid analogue, etretinate. Therefore, the non-esterified form of DCE (Ro 12-9933) may be more suitable for oncological treatment; however, the unreliable resorption of the oral formulation and the lack of an adequate parenteral form of DCE precludes its current use.

When the results of the present study are compared with those from a daily dose schedule described by Stüttgen (unpublished data), we found the main disadvantage of DCE, skin and mucosal toxicity, to be almost absent. Intermittent dosing of retinoids, therefore, seems to be a more appropriate way of oral retinoid administration to prevent skin and mucosal toxicity. The main disadvantage in using high, intermittent doses of retinoids by the oral route is attributable to the limitations of the gastrointestinal tract. Colburn and Gibson [5] suggest that 13-*cis* retinoic acid is more reliably absorbed. However, the differences they report in their study show no significant increases in the AUC_0^∞ from single doses over 160 mg, suggesting a limitation in the capacity of the gastrointestinal tract for high doses of 13-*cis* retinoic acid. This may also be applicable for other retinoic acid analogues.

The results of this study clearly demonstrate that increased doses of oral retinoids do not produce reliably increased plasma levels or improved uptake. Also, in view of the compromised gastrointestinal function in many cancer patients, it seems worthwhile to test a parenteral formulation of a suitable retinoic acid analogue for its efficacy in oncology. Parenteral administration also obviates the considerable first-pass effect that is very prominent in retinoic acid therapy [6]. A major problem in the use of parenteral retinoids is the fact that most retinoids are unstable in solution due to photosensitivity and poor solubility. The rather unpredictable results of retinoid treatment, for intance in patients suffering from myelodysplastic syndrome, might therefore be explained not only by primary resistance to retinoids but also by inadequate dosing. We conclude that further clinical studies with high-dose, oral retinoic acid analogues are not warranted in view of the unpredictable bioavailability.

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References

1. Bersha S, Rubinstein A, Paternit JR, Ngoc-Anh Le, Poliak SC, Heller B, Ginsberg N, Fleischmayer R, Brown WV (1985) Changes in plasma lipids and lipoproteins during isotretinoin therapy for acne. N Engl J Med 313: 981–985
2. Breitman TR, Selonick SE, Collins SJ (1980) Induction of differentiation of the human promyelocytic cell line (HL-60) by retinoic acid. Proc Natl Acad Sci USA 77: 2936–2940
3. Carter SK (1972) Study design principles for the clinical evaluation of new drugs as developed by the chemotherapy program of the National Cancer Institute. In: Staquet M (ed) The design of clinical trials in cancer therapy. Futura, Mount Kisco, NY
4. Clamon G, Chabot GG, Valeriote F, Davilla E, Vogel C, Gorowsky E, Birch R (1985) Phase I study and pharmacokinetics of weekly high-dose 13-*cis* retinoic acid. Cancer Res 45: 1874–1878
5. Colburn WA, Gibson DM (1985) Isotretinoin kinetics after 80 to 320 mg oral doses. Clin Pharmacol Ther 37: 411–414
6. Cotler S, Chen S, Macasieb T, Colburn WA (1984) Effect of route of administration and biliary excretion on the pharmacokinetics of isotretinoin in the dog. Drug Metab Dispos 12: 143–147
7. Daenen S, Vellenga E, Dobbenburg OA, von Halie MR (1986) Retinoic acid as antileukemic therapy in a patient with acute promyelocytic leukemia and *Aspergillus* pneumonia. Blood 67 (2): 559–561
8. Daudon M (1983) Renal toxicity of glafenine in man: renal stones and acute renal failure. Ann Biol Clin (Paris) 41 (2): 105–111
9. Dicken CH (1982) Retinoids: a Review. J Am Acad Dermatol 1: 541–552
10. Dion LD, Blalock JE, Gifford GE (1987) Vitamin A-induced density dependent inhibition of L-cell proliferation. J Natl Cancer Inst 58: 795–801
11. Fontana JA, Rogers JSD, Durham JP (1986) The role of 13-*cis* retinoic acid in the remission induction of a patient with acute promyelocytic leukemia. Cancer 57: 209–217
12. Gerber LH, Helfgott RK, Gross EG, Hicks JE, Ellenberg SS, Peck GL (1984) Vertebral abnormalities associated with synthetic retinoid use. J Am Acad Dermatol 10: 817–823
13. Gibaldi M, Perrier D (1982) In: Pharmacokinetics, 2nd edn, revised and expanded. Marcel Dekker, New York
14. Gold EJ, Mertelsman RH, Itrii LM, Gee T, Clarkson B, Moore MAS (1983) Phase I clinical trial of 13-*cis* retinoic acid in myelodysplastic syndromes. Cancer Treat Rep 67: 981–986
15. Hänni R, Hervoueth D, Busslinger A (1979) Determination of an aromatic retinoid and its main metabolite by high-performance liquid chromatography. J Chromatogr 162: 615–621
16. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD (1977) Assessment of reponse to therapy in advanced breast cancer. Eur J Cancer 13: 89–94
17. Hodiak E, Gadoth N, David M, Sandbank M (1986) Muscle damage induced by isotretinoin. Br Med J 293: 425–426
18. Hubert DD, Holiat SM, Smith WE, Baylouny RA (1983) Inhibition of transplanted carcinomas in mice by retinoids but not by vitamin C. Cancer Treat Rep 67: 1061–1065
19. Lotan R, Dennert G (1979) Stimulatory effects of vitamin A analogs on induction of cell-mediated cytotoxicity in vivo. Cancer Res 39: 55–58
20. Miller AB, Hoogstraten S, Staquet M, Winkler J (1981) Reporting results of cancer treatment. Cancer 74: 207–214
21. Pedersen H, Wolf H, Jensen SK, Lund F, Hansen E, Olsen PR, Sorenson BL (1984) Administration of a retinoid as prophylaxis of recurrent non-invasive bladder tumors. Scand J Urol Nephrol 18: 121–123
22. Rollman O, Vahlquist A (1983) Retinoid concentrations in skin, serum and adipose tissue of patients treated with etretinate. Br J Dermatol 109: 439–447
23. Rowland M, Tozer TN (1980) Clinical pharmacokinetics. Lea and Ferbiger, Philadelphia
24. Sporn MB, Dunlop NM, Newton DL, Smith JM (1976) Prevention of chemical carcinogenesis by vitamin A and its analogs (retinoids). Fed Proc 35: 1332–1338
25. Thein R, Lotan R (1982) Sensitivity of cultured human osteosarcoma and chondrosarcoma cells to retinoic acid. Cancer Res 42: 4771–4776

26. Thompson S, Stern PL, Webb M, Walsh FS, Engstrom W, Evans EP, Shi WK, Hopkins B, Graham CF (1984) Cloned human teratoma cells differentiate into neuron-like cells and other cell types in retinoic acid. J Cell Sci 72: 37 64
27. Vahlquist A, Loof L, Nordlinder H, Rollman O, Vahlquist C (1985) Different hepatotoxicity of two oral retinoids (etretinate and isotretinoin) in a patient with palmo-plantar psoriasis. Acta Derm Venereol (Stockh.) 65: 359–362
28. Zonnenberg BA, Neering H, Esch EP van der (1980) Het basale-cellen naevus syndroom. Ned Tijdschr Geneeskd 124: 1546–1549

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