Anti-Cancer Drugs 1999, **10**, pp. 295–302

Preclinical study

Comparative activity of the cyclopropylpyrroloindole compounds adozelesin, bizelesin and carzelesin in a human tumor colony-forming assay

Manuel Hidalgo, Elzbieta Izbicka, Cesario Cerna, Lionel Gomez, Eric K Rowinsky, Steven D Weitman and Daniel D Von Hoff

Institute for Drug Development, Cancer Therapy and Research Center, and University of Texas Health Science Center at San Antonio, San Antonio, TX 78245-3217, USA.

Adozelesin, bizelesin and carzelesin are synthetic cyclopropylpyrroloindole (CPI) analogs, a class of potent antineoplastic agents modeled on the antitumor antibiotic CC-1065, that specifically bind to the minor groove of DNA and preferentially alkylate AT-rich regions. These compounds were evaluated against fresh human tumors in a human tumor colony-forming assay (HTCFA) to assess and to compare their relative antitumor spectra, concentration–response relationships and schedule-dependence. Human tumor colony-forming units were treated with adozelesin and bizelesin at concentrations of 0.02, 0.1 and 0.5 ng/ml as a continuous exposure for 14 days, and to 0.2, 1.0 and 5.0 ng/ml as a 1 h exposure. Carzelesin concentrations were 0.04, 0.2 and 1 ng/ml as a continuous exposure, and 0.6, 3.0 and 15.0 ng/ml as a 1 h exposure. A response was scored if there was 50% or less colony survival. The three analogs had similar antitumor activity against colon carcinoma, kidney carcinoma and melanoma colony-forming units. Adozelesin also displayed activity against both breast and non-small cell lung carcinoma colony-forming units, and carzelesin was active against ovarian carcinoma colony-forming units. Significantly positive concentration–response relationships were apparent with all three agents. Responses increased from below 15% at the lowest concentration to above 45% at the highest concentration for the three drugs on all schedules ($p < 0.01$). At the highest concentration, the overall response rate was significantly higher ($p < 0.01$) with carzelesin on the continuous schedule (71%) compared to the 1 h schedule (46%). However, overall response rates for adozelesin and bizelesin were similar on both schedules (1 h/continuous: adozelesin, 67/58%; bizelesin, 49/44%), indicating that adozelesin and bizelesin are less schedule dependent than carzelesin in the HTCFA. These results demonstrate that the CPIs have broad-spectrum activity against human tumor colony-forming units in the HTCFA at very low concentrations, as well as differences with regard to schedule dependence which may help guide the optimal clinical development of these agents. [© 1999 Lippincott Williams & Wilkins.]

Key words: Adozelesin, bizelesin, carzelesin, human tumor colony-forming units.

Introduction

Adozelesin, bizelesin and carzelesin are potent synthetic cyclopropylpyrroloindole (CPI) analogs of the cytotoxic DNA binding antibiotic CC-1065.[1] CC-1065 is a fermentation-derived antitumor agent that binds in a non-intercalative manner in the minor groove of double-stranded DNA at regions rich in AT followed by covalent binding with the N^3 of adenine.[2–5] Although CC-1065 demonstrated moderate antitumor activity in murine models, it was not developed further because it produced irreversible hepatic and renal toxicities resulting in delayed lethality in mice (30 days after single i.v. bolus administration).[6] Structure-activity studies demonstrated that the alkylating moiety of CC-1065 was a CPI group and that an extended aromatic arm promoted binding to the minor groove of DNA.[7]

Because of the promising antitumor properties of CC-1065 in preclinical models and the finding that delayed death noted in mice with CC-1065 was associated with specific structural features which could be omitted from synthetic compounds, additional research activity was directed towards the

Presented in part at the Annual Meeting of the American Association for Cancer Research, New Orleans, 28 March–1 April 1998. MH is supported in part by grant 97/5161 from the Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Consumo, Spain, and is the recipient of an EORTC/NCI Fellowship Award.

Correspondence to E Izbicka, The Nordan Colon Research Laboratory, Cancer Therapy and Research Center, 7979 Wurzbach Road, San Antonio, TX 78229, USA.
Tel: (+1) 210 616-5892; Fax: (+1) 210 616-5948;
E-mail: eizbicka@saci.org

synthesis of CC-1065 analogs with improved therapeutic indices. Extensive exploration of CC-1065 analogs resulted in compounds with higher potency, greater absolute efficacy and lower toxicity than CC-1065.[6-8] Adozelesin, bizelesin and carzelesin were selected for clinical development based on their notable activity against a panel of murine tumors and human tumor xenografts, their high potency, and their desirable pharmaceutical properties. All three agents have an active CPI moiety in common attached to ligands of

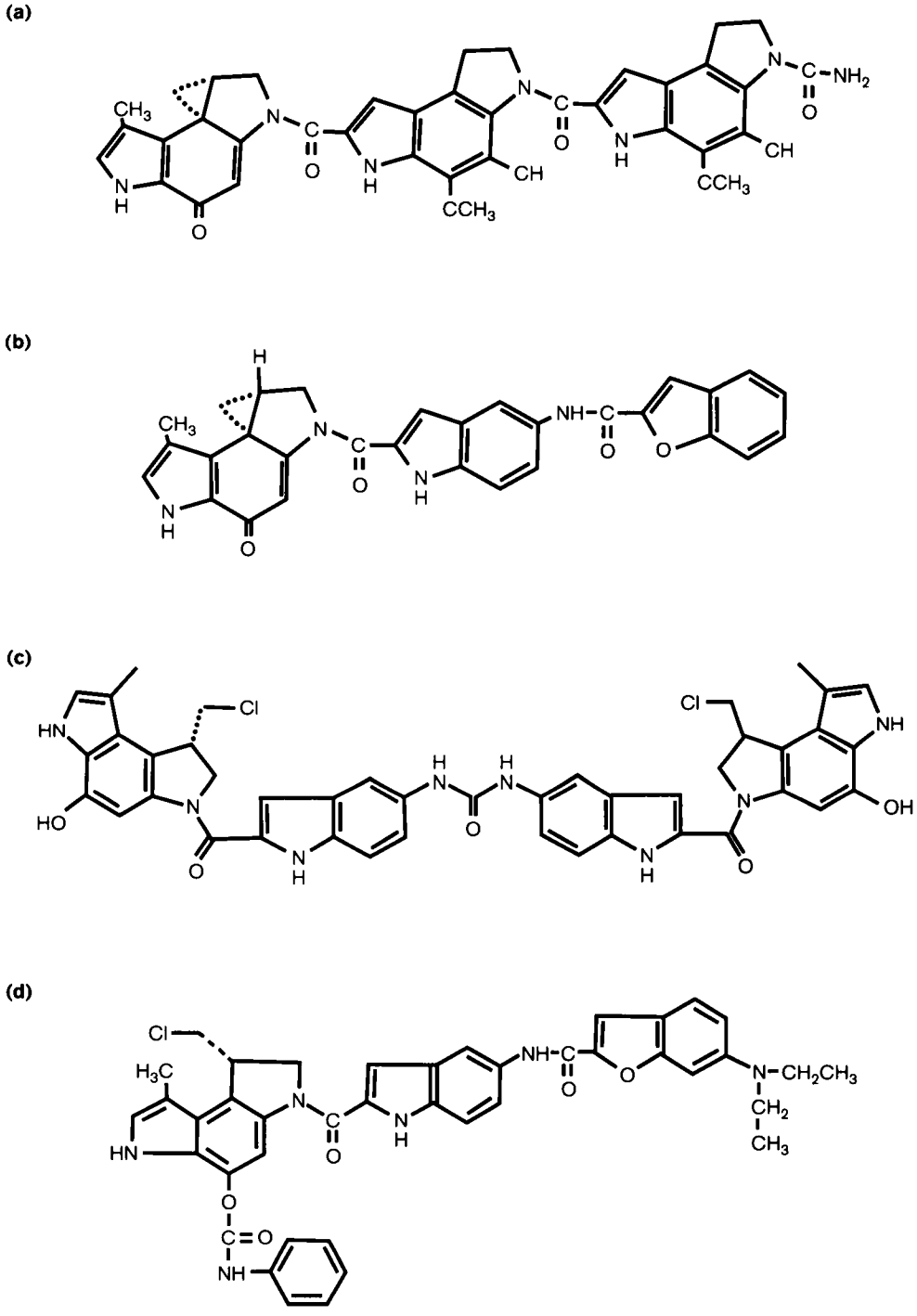

Figure 1. Chemical structure of (a) CC-1605 (the parent compound), (b) adozelesin, (c) bizelesin and (d) carzelesin.

appropriate length to optimize interactions with DNA (Figure 1). Adozelesin is a monofunctional alkylating agent and appears to target only specific adenine residues on double-stranded DNA. Carzelesin is a prodrug that requires hydrolysis of a phenylurethane substituent, followed by ring closure, to form the CPI-containing DNA-reactive compound. Similar to adozelesin, carzelesin is also a monofunctional alkylator. Bizelesin was synthesized in an effort to develop bifunctional analogs of CC-1065 capable of forming DNA interstrand cross-links. This compound is a prodrug of a bifunctional alkylating agent that contains two chloromethyl precursors of the CPI functional group. It undergoes conversion to the CPI form and alkylates the N^3 position of adenine in opposite DNA strands.

Preclinical studies have shown that these compounds possess remarkable activity against a wide range of *in vitro* and *in vivo* tumor models, and lack the delayed toxic effects reported with the parent compound CC-1065.[8-12] This report describes the activity, concentration-response relationships and schedule dependence of adozelesin, bizelesin and carzelesin against a panel of fresh human tumors taken directly from patients and plated in a human tumor cloning-forming assay (HTCFA).

Materials and methods

Collection of tumor cells

Tumor specimens were obtained using standard sterile techniques from patients undergoing diagnostic or therapeutic procedures. Specimens included solid tumors, malignant pleural effusions, ascites and bone marrow aspirates specimens. All patients gave written informed consent in accordance with federal and institutional guidelines. Solid tumors or lymph nodes were minced into 2-5 mm fragments in the operating room and immediately placed in McCoy's Medium 5A (Life Technologies, Grand Island, NY) containing 10% heat-inactivated newborn calf serum, 10 mM HEPES, 100 μg/ml penicillin and 90 μg/ml streptomycin (all Life Technologies) for transport to the laboratory. The fluids and bone marrow aspirate were placed in sterile containers containing 10 units of preservative-free heparin (O'Neill, Johns and Feldman, St Louis, MO)/ml of malignant fluid or marrow to prevent coagulation. Within 4 h, solid specimens were minced and passed repeatedly through metal sieves with 40 μm mesh (EC Apparatus, St Petersburg, FL) to obtain a single-cell suspension. When necessary, effusions were centrifuged at 150 *g* and passed through 25-gauge needles to obtain single-cell suspension. All specimens were washed twice in McCoy's medium containing 5% horse serum (Sigma, St Louis, MO), 10% heat-inactivated FCS (Hyclone, Logan, UT), 2 mM sodium pyruvate, 2 mM glutamine, 90 U/ml penicillin, 90 μg/ml streptomycin and 35 μg/ml L-serine (all Life Technologies) as previously described.[13-15] The viability of cell suspensions was determined on a hemocytometer with Trypan blue.

Drugs

Purified adozelesin (U-73,975), bizelesin (U-77,779) and carzelesin (U-80,244) were supplied by the Upjohn Company (Kalamazoo, MI).

In vitro exposure of tumor cells to drugs

Stock solutions of adozelesin, bizelesin and carzelesin were prepared in sterile, enriched Connaught Medical Research Laboratories (CMRL) Medium 1066 (Irvine Scientific, Irvine, CA). Aliquots of 0.5 ml of each stock solution were labeled and stored at −70°C. Aliquots were thawed for each new tumor sample tested. The final concentrations tested were: adozelesin and bizelesin; 0.2, 1.0 and 5.0 ng/ml as a 1 h exposure, and 0.02, 0.1 and 0.5 ng/ml as a continuous exposure; and carzelesin; 0.6, 3.0 and 15.0 mg/ml as a 1 h exposure, and 0.04, 0.2 and 1 ng/ml as a continuous exposure.

Culture of cells

Tumor cells were suspended in 0.35% agar in enriched CMRL 1066 medium (Life Technologies) supplemented with 15% heat-inactivated horse serum, penicillin (100 U/ml), streptomycin (2 mg/ml), glutamine (2 mM), insulin (3 U/ml), asparagine (0.6 mg/ml) and HEPES buffer (2 mM). Cells were plated in 35 mm Petri dishes in a top layer of agar and an underlayer of 0.3% agar to prevent growth of fibroblasts. Three plates were prepared for each data point. In the 1 h exposure studies, the cells were incubated with adozelesin, bizelesin and carzelesin in McCoy's medium, and then washed to simulate the disappearance of the drug from the body. In the continuous exposure condition, cells were combined with the drugs and plated as for the 1 h exposure and incubated for 14 days. The numbers of colonies (defined as more than 50 cells) formed in the three compound-treated plates were compared to the numbers of colonies formed in

the three untreated control plates and the percentage of surviving colonies at each concentration was calculated.

Quality control

To assure the presence of an adequate single-cell suspension on the day of plating, positive controls were used. For each tumor tissue sample tested, three positive control plates were set up to contain the cell poison orthosodium vanadate at 200 μg/ml, which should inhibit the growth of all clonogenic cells. If there was no effect of the positive control on colony formation, then the single-cell suspension on day 0 was poor (since orthosodium vanadate does not affect clumps) and the sample tested was considered non-evaluable. An evaluable test had an average of 20 or more colonies present on day 14 in the untreated control plates and less than 30% survival in the positive (orthosodium vanadate) control compared to the untreated control plates.

Data analysis and statistics

Results were expressed as the survival of tumor colony-forming units for a particular drug relative to its control expressed as percentages of the total cells. Response was defined as a growth inhibition of 50% or more in treated specimens compared to controls. The Mantel-Haenszel test for linear association was utilized to compare responses of the three drugs at each concentration for both schedules. Significant differences in responses at the same concentration for each drug at both schedules were expressed analyzed with a χ^2 test.

Results

In the 1 h exposure studies, a total of 162 specimens were treated with adozelesin, 128 with bizelesin and 167 with carzelesin; 72 (44%), 55 (43%) and 78 (47%), respectively, were evaluable according to the aforementioned criteria. Responses by tumor type at different drug concentrations are summarized in Table 1. There was a positive concentration-response relationship for all three compounds. Response rates were below 10% at the lowest concentrations, increased to 20–30% at the intermediate concentration and were above 45% at the highest concentration. These differences were statistically significant for all the drugs ($p < 0.01$, Mantel-Haenszel test for linear association). Drug activity was similar at the lowest and middle concentrations; however, at the highest drug concentration tested on this schedule, response rates were higher with adozelesin (67%) compared to

Table 1. Concentration-dependent inhibition of colony formation by adozelesin, bizelesin and carzelesin after 1 h exposure

Tumor type	No. specimens with inhibition/no. specimens evaluable								
	Adozelesin (ng/ml)			Bizelesin (ng/ml)			Carzelesin (ng/ml)		
	0.2	1	5	2	1	5	0.6	3.0	15.0
Bladder	0/2	0/2	1/2	0/2	0/2	1/2	0/2	0/2	0/2
Brain	0/1	0/1	0/1	0/1	0/1	0/1	0/2	0/2	1/2
Breast	0/13	2/13	7/13	1/12	3/12	3/12	1/13	1/13	5/13
Colon	2/9	3/9	6/9	1/8	3/8	6/8	1/8	2/8	4/8
Head and Neck	0/1	0/1	1/1	0/1	0/1	1/1	0/1	1/1	1/1
Kidney	0/7	1/7	4/7	0/7	2/7	5/7	0/10	5/10	6/10
Liver	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
NSCLC	0/12	2/12	8/12	1/10	1/10	1/10	1/14	2/14	5/14
SCLC	0/1	1/1	1/1	–	–	–	–	–	–
Lymphoma	0/1	1/1	1/1	0/1	1/1	1/1	0/1	0/1	0/1
Melanoma	2/16	8/16	12/16	0/7	2/7	5/7	1/9	3/9	6/9
Ovary	0/4	2/4	3/4	0/1	0/1	0/1	1/13	1/13	5/13
Gastric	0/2	1/3	2/3	1/2	1/2	2/2	0/3	3/3	3/3
Uterus	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Unknown primary	–	–	–	0/1	1/1	1/1	–	–	–
Total	4/71 (6%)	21/72 (29%)	48/72 (67%)	4/55 (7%)	14/55 (25%)	27/55 (49%)	5/78 (6%)	15/78 (19%)	36/78 (46%)

both bizelesin (49%) and carzelesin (46%) ($p < 0.05$).

Five tumor types (breast cancer, renal cancer, colon cancer, non-small cell lung cancer and melanoma) had more than five evaluable specimens treated with all three drugs at the highest concentration. The three analogs had similar activity levels in kidney carcinoma and melanoma specimens, ranging from 50 to 70%. Bizelesin had the lowest activity against breast cancer (27%) and non-small cell lung carcinoma (21%) compared with adozelesin (54 and 57%, respectively) and carzelesin (45 and 42%, respectively). Response rates against colon carcinoma colony-forming units were 71% with adozelesin and bizelesin, and 43% with carzelesin. However, the small number of specimens of each tumor type exposed to the three drugs precluded adequate statistical comparisons.

In the continuous exposure analysis (Table 2), 161 specimens were tested with adozelesin, 128 with bizelesin and 165 with carzelesin; 66 (41%), 52 (41%) and 70 (42%) were evaluable, respectively. Similar to the 1 h exposure, there was a significant concentration-dependent increase in the response rate. Responses were below 15% at the lowest concentration, increased to 30–40% at the intermediate concentration and were 44–71% at the highest concentration ($p < 0.01$, Mantel-Haenszel test for linear association). At the highest concentration studied, responses were significantly higher with carzelesin (71%) compared to adozelesin (58%) and bizelesin (44%) ($p < 0.05$, χ^2 test). The activity of the three analogs against specific tumor types in the continuous exposure studies are shown in Table 2. Again, group sizes were too small, precluding statistical comparisons.

The three drugs were similarly active (50–70%) against colon and kidney carcinoma colony-forming units. Adozelesin was also active against lung carcinoma colony-forming units (58%) and bizelesin showed activity against melanoma colony-forming units (57%). Carzelesin appeared superior in the continuous exposure. Except for lung cancer, response rates with carzelesin were higher than adozelesin and bizelesin for all tumor types. This was particularly evident in ovarian carcinoma specimens. The response rates with adozelesin and bizelesin were below 10%, whereas the response rate with carzelesin was 82%.

Adozelesin and bizelesin were schedule independent. At each concentration tested, the *in vitro* response rates were similar in the 1 h exposure and in the continuous exposure. In contrast, the response rates with carzelesin appeared schedule dependent; the overall response rate with this agent was higher in the continuous exposure compared to the 1 h exposure at all the concentrations tested (see tables). This exposure dependency was particularly evident at the higher concentration in that *in vitro* response rates were 46 and 71% with 1 h and continuous treatment, respectively ($p < 0.01$, χ^2 test). Response rates as a function of tumor type were also assessed. Adozelesin and bizelesin had a similar pattern of

Table 2. Concentration-dependent inhibition of colony formation by adozelesin, bizelesin and carzelesin after continuous exposure

Tumor type	No. specimens with inhibition/no. specimens evaluable								
	Adozelesin (ng/ml)			Bizelesin (ng/ml)			Carzelesin (ng/ml)		
	0.02	0.1	0.5	0.02	0.1	0.5	0.04	0.2	1.0
Bladder	0/2	1/2	1/2	0/2	1/2	1/2	1/2	0/2	1/2
Brain	0/1	0/1	0/1	0/1	0/1	0/1	0/2	1/1	1/1
Breast	0/11	1/11	4/11	1/10	2/10	3/10	1/10	2/10	5/10
Colon	0/8	2/8	7/8	1/7	3/7	4/7	0/7	2/7	5/7
Head and Neck	0/1	0/1	1/1	0/1	0/1	1/1	0/1	0/1	1/1
Kidney	0/7	1/7	4/7	1/7	3/7	5/7	1/10	5/10	8/10
Liver	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
NSCLC	1/12	3/12	4/12	1/11	1/11	1/11	3/13	7/14	10/14
SCLC	1/1	1/1	1/1	–	–	–	–	–	–
Melanoma	3/16	7/16	12/16	2/6	3/6	4/6	2/8	3/8	7/8
Ovary	0/3	1/3	2/3	0/2	0/2	0/2	2/12	6/12	9/12
Gastric	0/2	2/2	2/2	2/2	2/2	2/2	0/3	3/3	3/3
Uterus	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Unknown primary	–	–	–	0/1	1/1	1/1	–	–	–
Total	5/66 (8%)	19/66 (29%)	38/66 (58%)	8/52 (15%)	16/52 (31%)	23/52 (44%)	10/70 (14%)	29/71 (41%)	50/71 (71%)

activity on both schedules. For carzelesin, in contrast, activity was generally superior with the continuous exposure. This schedule dependency of carzelesin was particularly evident against ovarian cancer, as overall response rates were 36% with the 1 h exposure and 82% in the continuous exposure (p=0.04, χ^2 test).

Discussion

This report demonstrates that the three CPI analogs adozelesin, bizelesin and carzelesin exert concentration-dependent cytotoxic effects against a variety of fresh human tumor colony-forming units taken directly from patients. The HTCFA was initially developed to select the most appropriate chemotherapy agents for an individual patient's tumor.[13-15] More recently, this system has been utilized in several areas of drug development including gauging approximate drug plasma concentrations needed to achieve antitumor activity in patients, selecting the most appropriate schedule for clinical investigation and targeting tumor types for phase II trials.[16,17] The implementation of appropriate positive control plates,[15] as well as the simulation of drug concentration versus time exposures *in vitro* similar to those obtained *in vivo*,[18] has made this system more attractive as a method to evaluate the potential of a new agent.

This study shows a clear positive relationship between *in vitro* response rate and drug concentration both at 1 h and continuous exposures. The response rates at concentrations over 1 ng/ml were 3-fold higher than those at lower concentrations for all three drugs in both exposures. The differences were statistically significant for the three drugs using both exposure schedules. Previous work has revealed that *in vivo* therapeutic effects are observed in cloning assays only when plasma drug concentrations are obtained in the patients which are associated with significant inhibition of colony formation *in vitro*.[19] However, whether or not plasma levels in the active range in the HTCFA can be achieved in patients with these agents remain unknown. It should be noted that these agents are extremely potent at concentrations that are substantially lower than those that can be measured reliably, making characterization of their pharmacokinetics very difficult. Plasma concentrations have been recently measured in phase I studies of adozelesin and carzelesin using sensitive high-performance liquid chromatographic assays with a lower limit of quantification of 1-2 ng/ml.[20,21] In these studies, adozelesin plasma levels were undetectable at all dose levels below the maximum tolerated dose. At the recommended phase II dose of 150 $\mu g/m^2$, maximum plasma concentrations ranged from 3.4 to 21.3 ng/ml and decreased below the limit of quantitation by 20-30 min after the end of infusion, precluding a full characterization of the pharmacokinetics. In contrast, carzelesin could be detected in plasma at all dose levels and for longer periods of time, making it possible to fully estimate its pharmacokinetics. The AUC of carzelesin increased linearly with dose and was consistently over the highest concentration of 15 ng/ml tested in this study at dose levels over 210 $\mu g/m^2$, two dose levels below the maximum tolerated dose.[21] These data indicate that the plasma levels of these compounds in patients are consistently in the range noted to be active *in vitro* as determined in the cloning assay.

The overall response rates at the highest concentrations were similar for adozelesin and bizelesin on both schedules. However, the activity of carzelesin, particularly against ovarian cancer colony-forming units, appeared to be highly dependent on the duration of drug exposure. Other investigators have made observations which are consistent with the requirement of chemical or enzymatic conversion of carzelesin to its active form.[8] Since the half-life for this conversion in cell culture medium is approximately 40 min, a 1 h exposure period may be too short to achieve sufficient exposure of the tumor to the active species *in vitro*. In plasma from different species, the half-life for this metabolic activation ranges from 18 to 52 min.[8] Alternatively, the schedule dependency of carzelesin observed in this study may reflect a relationship between exposure to carzelesin and its cytotoxic activity. The data would suggest that adozelesin's and bizelesin's cytotoxic activity are unrelated to exposure time and optimal administration of these drugs should involve short infusion schedules.

This pattern of antitumor activity observed in the present study is similar to that observed in human tumor xenografts models in mice. Adozelesin was highly effective against a variety of human tumor xenografts implanted s.c. in nude mice, including CX-1 colon, LX-1 lung and CAK-1 clear cell renal carcinoma.[12] However, in contrast to its activity in the human cloning assay, this agent also had notable activity against the ovarian A2780 cell line.[12] Compared to the other two CPIs and to cisplatin and adriamycin, adozelesin was the most potent analog when evaluated against an *in vitro* panel of ten gynecologic cancer cell lines.[10] Bizelesin had also been demonstrated to delay the growth of CAK-1 renal, LOX-IMVI melanoma and HT29 colon cancer.[9] Melanomas were particularly sensitive to bizelesin, with nine of nine tumor-free survivors at 63 days.[9] Carzelesin also had activity

against a variety of human tumor xenografts including pediatric solid tumors[8,10] and demonstrated notable *in vivo* activity against ovarian cancer cell lines.[10]

The predictive value of these data regarding the ultimate clinical activities of the CPI analogs is not known at this juncture in their development. Adozelesin has completed phase I evaluation with various administration schedules including a brief i.v. infusion every 4 and 6 weeks, a brief infusion daily times 5 every 3 weeks, and a 24 h continuous infusion.[20-24] The dose-limiting toxicity in all of these studies has been delayed (2-3 weeks) myelosuppression, particularly thrombocytopenia. To date, antitumor activity had been noted in patients with malignant melanoma and soft tissue sarcoma.[20-24] Carzelesin has also completed phase I evaluations on two different schedules including a daily times 5 every 4 weeks schedule and a 10 min i.v. injection every 4 weeks schedule.[25] In both schedules, the principal dose-limiting toxicity was also delayed myelosuppression and a partial response was reported in one patient with hepatocellular carcinoma. Bizelesin is undergoing early phase I evaluation at this time.

In conclusion, this study demonstrated that the three CPI analogs adozelesin, bizelesin and carzelesin demonstrate prominent *in vitro* antitumor activity against common tumors types like colon cancer, melanoma, kidney cancer and lung cancer in colony-forming units. There was significantly greater antitumor activity at the higher concentrations for all the drugs and the results with carzelesin were improved using continuous exposure. Based on the results of this study, these agents may hold promise as anticancer drugs agents with potential for activity against a variety of common and refractory malignancies.

References

1. Mitchell MA, Kelly RC, Wicniensky NA, *et al.* Synthesis and DNA cross-linking by a rigid CPI dimer. *J Am Chem Soc* 1991; **113**: 8994-5.
2. Swenson DH, Li LH, Hurley LH, *et al.* Mechanism of interaction of CC-1065 (NSC 298223) with DNA. *Cancer Res* 1982; **42**: 2821-8.
3. Warpehoski MA. Stereoelectronic factors influencing the biological activity and DNA interaction of synthetic antitumor agents modeled on CC-1065. *J Med Chem* 1988; **31**: 3.
4. Hurley LH, Needham-VanDevanter DR. Covalent binding of an antitumor antibiotic in the minor groove of DNA. Mechanism of action of CC-1065 and the pyrrolol (1,4)benzodiazepines. *Acc Chem Res* 1986; **19**: 203-37.
5. Hurley LH, Reynolds VL, Swenson DH, Petzold GL, Scahill TA. Reaction of the antitumor antibiotic CC-1065 with DNA: structure of a DNA adduct with DNA sequence specificity. *Science* 1984; **226**: 843-4.
6. McGovren JP, Clarke GL, Pratt EA, *et al.* Preliminary toxicity studies with the DNA-binding antibiotic, CC-1065. *J Antibiot* 1980; **33**: 902-3.
7. Li LH, Wallace TL, DeKoning TF, *et al.* Structure and activity relationship of several model CC-1065 analogs. *Invest New Drugs* 1987; **5**: 329-37.
8. Li LH, DeKoning TF, Kelly RC, *et al.* Cytotoxicity and antitumor activity of carzelesin, a prodrug cyclopropylpyrroloindole analogue. *Cancer Res* 1992; **52**: 4904-13.
9. Cater CA, Waund WR, Li LH, *et al.* Preclinical antitumor activity of bizelesin in mice. *Clin Cancer Res* 1996; **2**: 1143-9.
10. Hightower RD, Sevin BU, Perras J, *et al. In vitro* evaluation of the novel chemotherapeutic agent U-73,975, U-77,779, and U-80,244 in gynecologic cancer cell lines. *Cancer Invest* 1993; **11**: 276-82.
11. Houghton PJ, Cheshire PJ, Hallman JD II, Houghton JA. Therapeutic efficacy of the cyclopropylpyrroloindole, carzelesin, against xenografts derived from adult and childhood solid tumors. *Cancer Chemother Pharmacol* 1995; **36**: 45-52.
12. Li LH, Kelly RC, Warpehoski MA, McGovern JP, Gebhard I, DeKoning TF. Adozelesin, a selected lead among cyclopropylpyrroloindole analogs of the DNA-binding antibiotic, CC-1065. *Invest New Drugs* 1991; **9**: 137-48.
13. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *J Clin Invest* 1977; **60**: 846-54.
14. Von Hoff DD, Sandbach JF, Clark GM, *et al.* Selection of cancer chemotherapy for a patient by an *in vitro* assay versus a clinician. *J Natl Cancer Inst* 1990; **82**: 110-6.
15. Shoemaker RH, Wolpert-Defillpes MK, Kern DH, *et al.* Application of a human tumor colony-forming assay to new drug screening. *Cancer Res* 1985; **45**: 213-53.
16. Hanauske AR, Degen D, Hilsenbeck SG, Bissery MC, Von Hoff DD. Effects of Taxotere and taxol on *in vitro* colony formation of freshly explanted tumor cells. *Anti-Cancer Drugs* 1992; **3**: 121-4.
17. Raymond E, Izbicka E, Soda H, Gerson SL, Dugan M, Von Hoff DD. Activity of temozolomide against human colony-forming units. *Clin Cancer Res* 1997; **3**: 1769-74.
18. Ali-Osman F, Giblin J, Dougherty D, *et al.* Application of *in vivo* and *in vitro* pharmacokinetics for physiologically relevant drug exposure in human tumor clonogenic cell assay. *Cancer Res* 1987; **47**: 3718-24.
19. Friedman, HS, Schold SC, Muhlbaier LH, Bjomsson TD, Bigner DD. *In vitro* versus *in vivo* correlations of chemosensitivity of human meduloblastoma. *Cancer Res* 1984; **44**: 5145-9.
20. Burris HA, Dieras VC, Tuncan M, *et al.* Phase I study with the DNA sequence-specific agent adozelesin. *Anti-Cancer Drugs* 1997; **8**: 588-96.
21. Tellingen O van, Punt CJA, Awada A, *et al.* A clinical pharmacokinetics study of carzelesin given by short-term intravenous infusion in a phase I study. *Cancer Chemother Pharmacol* 1998; **41**: 377-84.
22. Foster BJ, LoRusso PM, Poplin E, *et al.* Phase I trial of Adozelesin using the treatment schedule of daily $\times 5$ every 3 weeks. *Invest New Drugs* 1996; **13**: 321-6.

23. Fleming GF, Ratain MJ, O'Brien SM, *et al.* Phase I study of Adozelesin administered by 24-hour continuous intravenous infusion. *J Natl Cancer Inst* 1994; **86**: 368–72.
24. Shamdas GJ, Alberts DS, Mondlano M, *et al.* Phase I study of adozelesin (U-73,975) in patients with solid tumors. *Anti-Cancer Drugs* 1994; **5**: 10–4.
25. Punt CJ, Awada A, Janssens A, *et al.* Carzelesin (U-80, 244): toxicity patters and pharmacokinetic data of two phase I studies. *Ann Oncol* 1996; **7** (suppl 1): 30.

(Received 3 December 1998; accepted 17 December 1998)