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ORIGINAL ARTICLE

Corticorelin acetate, a synthetic corticotropin-releasing factor with preclinical antitumor activity, alone and with bevacizumab, against human solid tumor models

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Abstract
Purpose Corticorelin acetate (CrA) is a synthetic form of corticotropin-releasing factor undergoing clinical trials in the treatment of peritumoral brain edema (PBE). We sought to investigate preclinically its potential as an antitumor agent against human solid tumors and to assess its ability to enhance the therapeutic activity of bevacizumab (BEV) in these same models.
Methods The in vivo efficacy of CrA as a single agent and in combination with the antiangiogenic agent, BEV, was examined in two preclinical human tumor models, the MX-1 breast and Colo-205 colon carcinomas. These models were selected based on their known sensitivity to BEV and were tumor types in which BEV has been approved for clinical use. The corneal micropocket assay was also performed to assess the antiangiogenic activity of CrA relative to BEV. The exposure level of CrA in the mouse using a typical preclinical regimen was measured so as to compare it to reported clinical exposure levels.
Results CrA was active as a single agent in the MX-1 breast carcinoma, but did not exhibit statistically significant activity as a single agent in the Colo-205 colon carcinoma under the doses and schedules used in the study. When BEV, which was active or near active in both the MX-1 and Colo-205 models, was administered concomitantly with CrA, therapeutic outcomes were observed that were significantly better than those obtained using either monotherapy. These therapeutic potentiations using CrA plus BEV were obtained in the absence of any observable increase in toxicities. CrA was active in the corneal micropocket assay, producing a substantial (>70%) inhibition of neovascularization. A representative CrA regimen in mice produced an exposure within eightfold of human exposure determined at one-half the current clinical dose.
Conclusions The application of CrA for the treatment of PBE likely involves its activity as an antiangiogenic agent, which may be one possible mechanism to explain its observed preclinical antitumor activity. That activity, as well as its ability to provide an enhanced therapeutic outcome when given in conjunction with BEV in the absence of increased toxicity, supports the use of CrA clinically as other than a replacement therapy for dexamethasone in PBE.

Keywords Corticorelin acetate · Xerecept® · Bevacizumab · Avastin® · Corticotropin-releasing factor · Antitumor

Introduction

Corticorelin acetate (Xerecept®, CrA) is a synthetic peptide formulation of the endogenous neurohormone, corticotropin-releasing factor (CRF). The 41-amino acid sequence of CrA is identical to that of the human hormone, and it is being developed as an alternative to dexamethasone in the treatment of peritumoral brain edema (PBE). Patients are typically debilitated by the effects of PBE associated with brain tumors. The cause of PBE is believed to result in-part from the leakage of edematous brain tumor fluid from abnormal tumor vasculature into the surrounding tissue [1, 2]. The mechanism(s) of action by which CrA exerts its

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beneficial effect in the therapy of PBE has not been elucidated, but two factors appear to be relevant: decreased vascular leakage and preserved integrity of the endothelial cells, which ultimately helps to maintain the blood–brain barrier [3, 4].

CrA appears to mediate its activity through two subtypes of G protein -coupled receptors: CRF Receptor-1 (CRFR1) and CRF Receptor-2 (CRFR2) [5]. These CRF receptors (CRFRs) are widely distributed in the central nervous system, peripheral tissues, and some types of human tumors [6, 7]. It is well known that CRFR2 plays a critical role in the tonic inhibition of adult neovascularization [8]. Moreover, it has been found that CRFR2 agonism inhibited the growth of hepatocellular carcinoma and reduced tumor microvessel density in nude mice [9]. Hepatoma cells do not express CRFRs, whereas associated blood vessels express CRFRs, mainly CRFR2 [9]. As part of this investigation, we included the corneal micropocket assay to help characterize the antiangiogenic potential of CrA and determine its mechanism of action [10].

Ongoing clinical trials involving nearly 200 patients that have received CrA indicate that subcutaneous (sc) administration of the drug, often for extended periods, is well tolerated [11–13]. The evolving clinical efficacy and safety data supports the use of CrA as a dexamethasone-sparing treatment (if not an alternative to dexamethasone) for the management of symptomatic PBE [14].

Preclinical studies involving the use of CrA in the treatment of mice bearing orthotopically implanted human brain tumors yielded encouraging positive data [15, 16]. Additionally, based on data to be described herein, we have observed that CrA has activity as an antiangiogenic. If CrA and another antiangiogenic compound with known antitumor activity had complimentary mechanisms of action, would their use in combination provide an enhanced therapeutic benefit? Thus, our objectives in the present studies were to investigate further, preclinically, the potential of CrA as an antitumor agent in non-glioma models and to evaluate CrA in combination with bevacizumab (BEV, Avastin®), a well known antiangiogenic [17]. For these purposes, the tumor models selected were those known to be sensitive to BEV therapy and tumor types in which BEV has been approved for clinical use.

Materials and methods

Animals

Female, athymic nude mice (8–12 weeks old) were purchased from Harlan Laboratories and were used for all studies. Animals were maintained in filter top cages in Thoren units. All animal procedures conformed to the Piedmont Research Center (Piedmont), Morrisville, NC, Institutional Animal Care and Use Committee guidelines. Additionally, all the mice were maintained according to National Institutes of Health guidelines and the Piedmont animal facility is approved by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

The corneal micropocket assay was also performed at Piedmont Morrisville, NC. Female C67BL/6 mice, approximately 6 weeks old when used, were purchased from Charles River Breeding Laboratories (Wilmington, MA) and housed in microisolators.

The pharmacokinetic study was conducted at Farmington Pharma Development Corporation, and the Hartford Hospital, Hartford, CT. The animal facility used for the study is AAALAC accredited and is registered with the Department of Agriculture. Male, athymic nude mice were purchased from Harlan Laboratories (Indianapolis, IN) and were 4–5 weeks old when used.

Compounds

CrA was provided in clinical vials with dilutions made in saline. It was prepared for injection fresh each morning and stored at 4°C in anticipation of the afternoon injection. Clinical vials of BEV were provided by Piedmont and dilutions made in saline on the day of use. Vascular endothelial growth factor (VEGF) was obtained from R & D Systems (Minneapolis, MN).

Tumor xenografts and implantation

The following human tumor xenografts maintained by Piedmont were used: MX-1 breast carcinoma and Colo 205 metastatic colon carcinoma.

Mice were implanted sc in the flank with approximately 1 mm^3 tumor fragments obtained from donor passage mice (MX-1), or with 1×10^6 tissue culture-derived Colo-205 cells implanted in 50% Matrigel (BD Biosciences). When tumors were generally between 80 and 240 mg (mm^3), tumored mice were distributed to control and treatment sets such that each set's mean tumor size was within 10% of the overall mean tumor size of all mice included in the experiment.

Subcutaneous tumor measurement

Sc tumors were measured twice weekly with hand-held vernier calipers. Tumor volumes were calculated by the formula: $[(\text{width})^2 \times (\text{length})]/2 = mm^3$ tumor volume. Cubic mm was considered equivalent to mg. Measurements were made twice weekly until the completion of each

experiment. Each tumored mouse was killed when it achieved the predetermined (see Sect. "Drug therapy", below) tumor target size.

Drug therapy

For the MX-1 tumor xenograft experiment, the initial day of treatment for all groups ("start day") was Day 17 post-tumor implant. Individual tumors ranged from 63 to 245 mg, with a mean of approximately 132 mg. For the Colo-205 experiment, the start day for all groups was Day 20 post-tumor implant. Individual tumors ranged from 88 to 221 mg, with an approximate mean of 166 mg.

CrA was administered at two dose levels, 0.1 and 0.2 mg/kg/injection, sc, twice daily (bid), every 12 h. BEV was administered at a dose of 5 mg/kg/inj, ip, twice weekly, beginning on the same "start day" as CrA. All treatments were continued until each individual animal achieved its tumor target size. An exception is in the combination treatment group in the Colo-205 study, which was terminated on Day 121. The predetermined tumor target size was 1 gm (1,000 mm^3) for mice in the Colo-205 experiment and 1.5 gm (1,500 mm^3) for mice in the MX-1 experiment. Thus, treatment durations for each group, and even individual mice within a group, differed according to the time it took for a given mouse to grow a tumor of target size. Treatment regimens and group sizes are summarized in Table 1. When a mouse received both BEV and CrA on the same day, the two drugs were given at the same prescribed times as for mice receiving solo treatments.

Mice were weighed twice weekly, individually, and drugs were administered on a per kg basis according to the last weight recorded for each mouse to be injected. Injection volumes were delivered in 0.01 ml/gm of body weight.

Table 1 Sc-implanted human tumor xenograft experimental designs

Tumor model	*n*	CrA[a] (mg/kg/inj, sc, bid to end)	BEV[a] (mg/kg/inj, ip, 2×/week to end)
MX-1 Breast	9	–	–
	8	0.1	–
	8	0.2	–
	8	–	5
	8	0.2	5
Colo 205 Colon	10	–	–
	10	0.1	–
	9	0.2	–
	10	–	5
	10	0.2	5

[a] Corticorelin acetate (CrA) and Bevacizumab (BEV) were administered twice daily (bid) or twice weekly (2×/week) until each group of mice achieved its median time to tumor target size of 1 gm (Colo-205) or 1.5 gm (MX-1)

Toxicity assessment

Body weight

The average body weight of each treatment group and vehicle-treated control set was determined prior to the first dose on the day of treatment initiation. Average body weights were determined daily for the first 5 days, then 2×/week until study end. The difference in average body weights (before versus during and post-treatment) was used to estimate treatment-related toxicity. A loss of average body weight greater than 20% was considered to be indicative of an excessively toxic treatment.

Deaths

A treatment group with more than one death attributable to drug toxicity (e.g., a death occurring prior to any death in the relevant control set accompanied by excessive weight loss) was considered to have had an excessively toxic treatment.

Efficacy assessment

Tumor growth delay

Therapeutic results are presented in terms of (a) tumor growth delay reflected by the relative *median* time for drug-treated (*T*) mice versus vehicle-treated control (*C*) mice to achieve tumor target size (i.e., T–C in days), and (b) cures. A mouse was considered "cured" if the tumor was not palpable or less than 13.5 mg (mm^3) at the site of tumor inoculation at the termination of an experiment.

Activity was defined as a delay in tumor growth (T–C) of not less than 3.32× the control group's tumor volume doubling time (TVDT) (determined between tumor target size and ½ tumor target size), accompanied by a statistical difference (of $P < 0.05$) using a log rank test [18]. When the T–C value was divided by 3.32 × TVDT,

$$LCK = T - C/(TVDT)(3.32)$$

the result was a measure of tumor growth delay caused by tumor cell killing and growth inhibition and was referred to for convenience as log cell kill (LCK). An LCK of ≥1.0 was considered indicative of activity.

Mice with tumors, which exceeded their tumor target size were killed. When determining median tumor weights (used for graphing), the tumors of killed mice were

included in the calculation of group median tumor weights (at their final recorded tumor weight).

Corneal micropocket assay

C57/BL/6 mice were anesthetized with 90 mg/kg sodium pentobarbital on Day 0, and the right cornea of each mouse was subjected to keratotomy under a dissecting microscope. A pellet containing VEGF (129 ng), or excipient without VEGF, was implanted in an intrastromal pocket in the cornea.

On Day 1 of the study, mice were sorted into groups ($n = 3$ or 8) and treatments were initiated. A mock pellet group without VEGF (Set 1, $n = 3$) and a group with VEGF (Set 2, $n = 3$) were included to monitor VEGF-induced vessel growth. The vehicle control group received phosphate buffered saline, sc, bid × 7 (Set 3). The positive reference group received BEV, ip, qd × 7, at 5 mg/kg/inj (Set 4). The two remaining groups received CrA sc, bid × 7 at 0.1 mg/kg/inj (Set 5) or 0.2 mg/kg/inj (Set 6). All drug doses were administered as 0.01 ml/gm of body weight.

On Day 8, mice were anesthetized with 90 mg/kg sodium pentobarbital for the assessment of neovascularization. Semi-quantitative characterization of neovascularization was achieved from two perspectives. New vessel length (VL) was measured with a linear reticle on a slit lamp. A circumferential zone of new vessel formation was measured on the basis of clock hours (CH) where one CH = 30 degrees. Neovascular area was calculated using the formula for half an ellipse: area (mm^2) = $0.2\pi \times VL \times CH$. The Kruskal–Wallis non-parametric test with Dunn's post hoc multiple comparison test was employed to assess significance of the difference between the mean area of neovascularization of two groups [19].

Pharmacokinetic study

Athymic nude mice received 0.1 mg/kg/inj of CrA, sc, bid (10 h apart for 3 days). Drug administration was performed using non-fasted mice with access to food and water ad libitum. Mice were dosed on an individual body weight basis. Blood was obtained at the following time points (relative to 1st dose on Day 3) 0.25,1, 2, 4, 10, 10.25, 11, 12, 14 and 24 h. Three animals were used at each collection time. Blood samples were collected via cardiac puncture under CO_2 anesthesia, and plasma was prepared by centrifugation. Non-compartmental pharmacokinetic analysis was performed using WinNonlin Professional v 5.0.1 (Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration of compound (C_{max}) and the time at C_{max} (T_{max}) were determined from the observed values. The elimination half-life ($t_{1/2}$) and the area under the CrA concentration–time curve from time zero to the last quantifiable concentration of CrA (AUC_{0-t}) were calculated using WinNonlin. Concentrations below the lower limit of quantification were assigned a value of zero for the calculation of mean values.

Results

A summary of the experimental designs of the two efficacy experiments is shown in Table 1, and the results of those studies are presented in Table 2.

Table 2 Antitumor activities of CrA and BEV versus Sc human tumor xenografts

Tumor	CrA (mg/kg/inj, sc, bid)	BEV (mg/kg/inj,ip, 2×/week)	Median time to target (days[a])	T–C (days)	Notes[b]
MX-1	–	–	19.8		
	0.1	–	23.0	3.2	NS
	0.2	–	35.1	15.3	1.0 LCK ($P < 0.02$)
	–	5	37.1	17.2	1.1 LCK ($P < 0.002$)
	0.2	5	72.6	52.8	3.3 LCK ($P < 0.001$) [and vs. monotherapies ($P < 0.03$)]
Colo 205	–	–	29.9	–	
	0.1	–	30.7	0.8	NS
	0.2	–	36.3	6.4	NS
	–	5	72.6	42.7	0.9 LCK ($P < 0.001$)
	0.2	5	>121[c]	91.1	>1.9 LCK ($P < 0.001$) [and $P < 0.01$ vs. monotherapies]

[a] Expressed as days post-initiation of treatment (Day 1 corresponds to the following days post-tumor implant : Day 17 for MX-1, and Day 20 for Colo 205)

[b] *NS* not significant. LCK values indicate level of activity by this parameter (accompanied by statistical significance as indicated vs. control set); other comparisons are specified

[c] Experiment terminated on Day 121 post-treatment

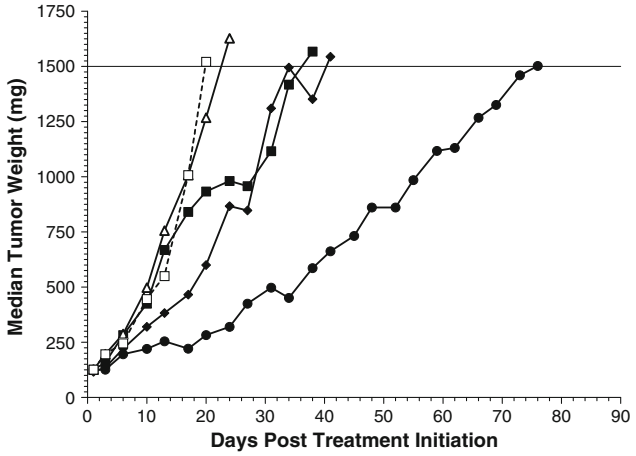

Fig. 1 Effect of BEV and CrA, individually and in combination, versus the MX-1 human breast carcinoma xenograft. (*Empty square*) Control; (*Empty triangle*) CrA, 0.1 mg/kg/inj, bid, sc; (*Filled square*) CrA, 0.2 mg/kg/inj, bid, sc; (*Filled diamond*) BEV, 5 mg/kg/inj, 2×/week, ip; (*Filled circle*) CrA, 0.2 mg/kg/inj, bid, sc and BEV, 5 mg/kg/inj, 2×/week, ip

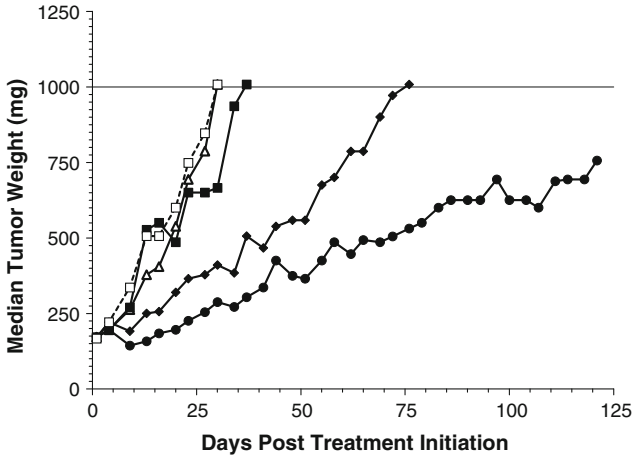

Fig. 2 Effect of BEV and CrA, individually and in combination, versus the Colo-205 human colon carcinoma xenograft. (*Empty square*) Control; (*Empty triangle*) CrA, 0.1 mg/kg/inj, bid, sc; (*Filled square*) CrA, 0.2 mg/kg/inj, bid, sc; (*Filled diamond*) BEV, 5 mg/kg/inj, 2×/week, ip; (*Filled circle*) CrA, 0.2 mg/kg/inj, bid, sc and BEV, 5 mg/kg/inj, 2×/week, ip

MX-1 human breast carcinoma model

Both CrA and BEV were active in mice bearing MX-1 tumors. The higher dose of CrA and BEV produced nearly identical delays in tumor growth of 15–17 days T–C. Considering the TVDT in this experiment of 4.8 days, these delays were equivalent to 1.0 and 1.1 LCK, respectively. These levels of activity were statistically significant ($P < 0.02$–0.002). As shown in Fig. 1, when BEV was combined with CrA, the extent of tumor growth delay was greatly increased reflecting 3.3 LCK ($P < 0.001$). The enhanced combination effect was statistically different ($P < 0.03$) than either result associated with the monotherapies. No treatment group in this experiment was found to be excessively toxic by the criteria described in the Sect. "Materials and methods".

Colo-205 human colon carcinoma model

CrA did not exhibit statistically significant activity against the Colo-205 carcinoma as a single agent under the doses and schedules used in this study. BEV alone produced a nearly 43-day T–C, or given the 14.6-day TVDT in this experiment, 0.9 LCK which is a borderline result ($P < 0.001$). The combination of CrA with BEV was highly active. Mice receiving this treatment had not reached tumor target size as of Day 121 when the experiment was terminated. The delay in tumor growth of ~91 days (~1.9 LCK) compared to control mice was statistically significant ($P < 0.001$). Additionally, this tumor growth delay also reflected a ~48-day improvement compared to the BEV-only treatment group ($P < 0.01$). These effects can be seen in Fig. 2. No treatment group in this experiment was found to be excessively toxic by the criteria described in the Sect. "Materials and methods".

Corneal micropocket assay

A summary of the assay results is provided in Table 3. Groups 1 and 2 were negative and positive controls, respectively, for pellet utility. Group 3 received VEGF and only the vehicle used for CrA. As expected, there was no neovascularization seen in group 1. Group 2 (no treatment) and group 3 (vehicle only) had mean neovascularization areas of 1.89 and 1.60 mm^2, respectively, indicating an induction of angiogenesis toward the VEGF pellet. Group 4 received VEGF plus BEV (5 mg/kg/inj, ip, qdx7) and exhibited complete inhibition of neovascularization; this effect was highly significant when compared to group 3 (vehicle control group) ($P < 0.001$).

Groups 5 and 6 were also implanted with pellets containing VEGF. Group 5, CrA at 0.1 mg/kg/inj, and group 6, CrA at 0.2 mg/kg/inj, exhibited mean neovascularization areas of 1.23 and 0.47 mm^2, respectively, which are 77 and 29% of that seen in group 3. The inhibition of neovascularization seen in group 6 was significant compared to group 3 ($P < 0.01$).

Representative pictures of mouse cornea from groups 3, 4, 5 and 6 in this assay are presented in Fig. 3 to highlight the antiangiogenic effects of both BEV and CrA.

All treatments were acceptably tolerated with negligible or no bodyweight losses observed.

Table 3 Effect of CrA and BEV on VEGF-induced neoangiogenesis in mouse cornea

Group	*n*	Treatment[a]	Area (mm^2) of neovascularization, Mean ± SEM	% of positive control (Group 3)	Statistical significance?
1	3	None	0.00 ± 0.00	–	–
2	3	VEGF only	1.89 ± 0.19	–	–
3	8	VEGF + vehicle	1.60 ± 0.10	100	–
4	8	VEGF + BEV 5	0.00 ± 0.00	0	$P < 0.001$
5	8	VEGF + CrA 0.1	1.23 ± 0.11	77	NS
6	8	VEGF + CrA 0.2	0.47 ± 0.06	29	$P < 0.01$

Abbreviations used: *SEM* standard error of the mean, *BEV* bevacizumab, *CrA* Corticorelin acetate, *NS* not significant ($P > 0.05$). Group 3 served as the positive vehicle-treated control group. Statistical significance was assessed using a Kruskal–Wallis Dunn's test [18]

[a] BEV and CrA treatments are expressed in mg/kg/inj. BEV was administered ip, qdx7. CrA was administered sc, bid, qdx7. All treatments began one day post-insertion into the corneal pocket of a pellet containing 129 ng VEGF (groups 2–6)

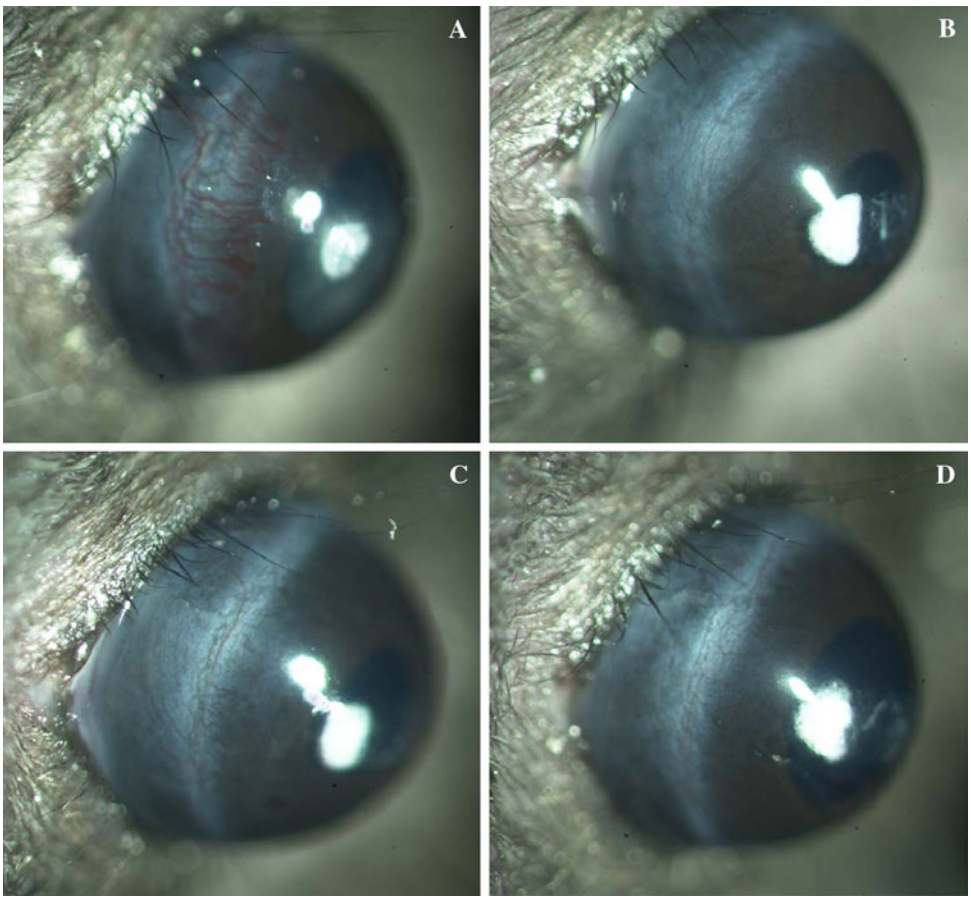

Fig. 3 Effect of CrA and BEV on angiogenesis in the corneal micropocket assay. Representative photographs of corneas of mice injected with CrA vehicle only (**a**); BEV, 5 mg/kg/inj, ip, qdx7 (**b**); CrA, 0.1 mg/kg/inj, sc, qdx7 (**c**) and CrA, 0.2 mg/kg/inj, sc, qdx7 (**d**) after implanting hydron pellets containing 129 ng of VEGF. The effects of these treatments are summarized in Table 3

Mouse pharmacokinetic data

Mean C_{max} value of 241.7 ng/ml was observed at mean T_{max} time of 10.25 h. The early T_{max} value (occurring at 0.25 h after the second daily dose) indicates that CrA was rapidly absorbed from the subcutaneous site of administration. The T_{max} values are also consistent with expectations for bid administration—occurring at the first sampling point after the second daily dose. Additionally, the C_{max} following the second daily dose was very similar to that found after the first daily dose—pharmacokinetics consistent with the short plasma half-life of CrA in the mouse.

Plasma level declined rapidly in mice receiving 0.1 mg/kg/inj, sc, bid of CrA but remained quantifiable for 14 h following the first injection. The apparent mean plasma

half-life ($t^{1/2}$) value was 0.57 h. The mean AUC_{0-t} value was 30,474 ng-min/ml.

Discussion

CrA was active as monotherapy against the human breast carcinoma, MX-1. It did not exhibit statistically significant activity against the Colo-205 colon carcinoma at the doses and treatment schedules tested. BEV alone achieved about 1 LCK in both the MX-1 breast and Colo-205 colon models, demonstrating BEV's antitumor activity as previously described in several human cancer xenograft models [20–22]. When human tumor xenografts grown in nude mice respond to BEV, or its murine antibody equivalent, a maximum therapeutic effect is reached at about 5 mg/kg/inj, twice weekly, ip, or less [20–26].

Mice bearing either Colo-205 or MX-1 tumors benefited from the combination therapy of CrA and BEV. This treatment provided a greater delay in tumor growth than was achieved with the best concomitant monotherapy. Such potentiation occurred whether the tumor model was susceptible (MX-1) or not (Colo-205) to CrA alone. In addition, the enhanced therapeutic outcomes were obtained in the absence of any observable toxicities.

The role of CRFR2 in tumor formation and angiogenesis is of continued interest. For example, activation of CRFR2 was observed to suppress angiogenesis and rearrange vasculature [8]. Additionally, activation of CRFR2 inhibited the proliferation of rat smooth muscle cells in vitro, subsequent to VEGF suppression, and reduced capillary tube formation in collagen gels [27]. It was also reported that CRFR2 agonism inhibited hepatocellular carcinoma tumor angiogenesis in vitro and reduced tumor microvessel density in vivo [9]. Conducting in vitro studies with human umbilical endothelial cells, Tjuvajev et al. [28] demonstrated that VEGF-induced proliferation could be inhibited with CRF. Thus, the observed vascular and antiproliferative effects, in both endothelial and tumor cells, may explain the antineoplastic properties of CRF in vivo.

CrA administration (0.2 mg/kg/inj) resulted in a significant suppression of neovascularization. Our findings demonstrate that CRF has antiangiogenic effects in vivo. The positive results obtained in the current study provide a possible explanation as to a mechanism of action, but we are still engaged in a search for other mechanisms to explain the observed antitumor activities of CrA.

Lastly, we investigated the exposures of CrA achieved in mice when using a dosing regimen that had been evaluated in the antitumor tests, 0.1 mg/kg/inj. The AUC level attained may be viewed relative to that found in a human pharmacokinetic evaluation [29]. Following once daily sc administration of 1.2 mg of CrA to healthy volunteers for three consecutive days, the mean $AUC_{0-\infty}$ level measured after the third day of treatment was 3,846 ng.min/ml. This value may be compared to the mean exposure obtained in mice receiving 0.1 mg/kg CrA, bid, sc (AUC_{0-t} of 30,474 ng.min/ml). The mouse exposure was 7.9-fold greater than the human exposure for these selected dose levels. Although the human doses currently being investigated in ongoing clinical trials (e.g., 1 mg CrA, sc, bid), about twice the amount used in the aforementioned pharmacokinetic assessment, were intended to control edema and not to serve as antitumor treatments *per se*, it is conceivable that CrA dose escalation in humans for the purpose of anticancer therapy could be safely tolerated and would be synonymous with even greater exposure levels.

Exogenous human CRF administered parenterally produces dose-dependent release of adrenocorticotropic hormone (ACTH) and increased circulating levels of glucocorticosteroids in the mouse, rat, dog, monkey and human [29]. However, only modest elevations of plasma levels of ACTH and cortisol were observed in humans [29, 30].

In summary, CrA, a synthetic formulation of human CRF, has further demonstrated its effectiveness as an antitumor agent. It has been shown to significantly increase the lifespan of mice implanted with two different human solid tumors. Furthermore, in both of those tumor models, the combination of CrA with the antiangiogenic drug, BEV, produced a therapeutic outcome superior to that found using either of the two agents alone. On the basis of relevant assay data presented, antiangiogenesis remains one possible mechanism of action for the observed antitumor effects of CrA. The exposure (AUC) in mice associated with a CrA dose about one-half the therapeutic level was nearly eightfold greater than the exposures found in humans administered about one-half the current clinical dose of CrA for the treatment of PBE. Since these doses are well tolerated clinically [11–13], dose escalation for the treatment of tumors remains feasible. Additional preclinical antitumor and mechanism of action studies are underway.

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