Cancer Chemother Pharmacol (2006) 57: 559–568
DOI 10.1007/s00280-005-0078-4

ORIGINAL ARTICLE

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Phase I/II dose escalation study of angiotensin 1-7 [A(1-7)] administered before and after chemotherapy in patients with newly diagnosed breast cancer

Received: 17 March 2005 / Accepted: 14 July 2005 / Published online: 12 August 2005

Abstract *Purpose*: Multilineage cytopenias occur following myelosuppressive chemotherapy. Most hematopoietic agents differentiate along a single lineage and fail to prevent progressive cytopenias. Angiotensin 1-7 [A(1-7)] is a hematopoietic agent that stimulates the proliferation of multipotential and differentiated progenitor cells in cultured bone marrow and human cord blood. The purpose of this study was to determine the optimal biologic dose and the maximum tolerated dose of A(1-7). *Experimental design*: This study determined the safety and activity of A(1-7) following chemotherapy in patients with breast cancer. Toxicity was assessed by administering A(1-7) daily for 7 days followed by a 7-day washout prior to the first cycle of chemotherapy. Beginning 2 days after chemotherapy and continuing daily for at least 10 days, fifteen patients received five different A(1-7) doses and five patients received filgrastim as a comparator group over three cycles of chemotherapy. *Results*: No dose-limiting toxicity was observed following A(1-7). The frequency of adverse events was slightly lower in A(1-7) than in filgrastim patients. No patient required a chemotherapy modification due to hematologic toxicity. There was an apparent differential dose-response sensitivity of the various lineages to A(1-7). At a dose of 100 μg/kg, A(1-7) reduced the frequency of grade 2–4 thrombocytopenia, anemia, and grade 3–4 lymphopenia as compared to filgrastim. *Conclusion*: These data suggest that A(1-7) may be beneficial in attenuating multilineage cytopenias following chemotherapy at a dose of 100 μg/kg per day.

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Keywords Thrombocytopenia · Lymphopenia · Myelosuppression · Stomatitis · Alopecia

Introduction

Delivery of optimal dosing of cytotoxic chemotherapy is often limited by myelosuppression. Depending on the chemotherapy regimen, multilineage cytopenias can be severe, leading to treatment delays and the serious comorbidity of infection [39], anemia [15, 37], and/or bleeding [1]. Myelosuppression is associated with a reduction in both undifferentiated and differentiated hematopoietic progenitor cells in the bone marrow [13, 29, 40]. The aplastic phase of myelosuppression is associated with normal or even elevated endogenous growth factors rather than a deficiency [9, 16]. In some cases, decreased responsiveness to these endogenous growth factors has been reported [24]. Correction of cytopenias will occur without exogenous growth factor support once the bone marrow progenitor pool is reconstituted. Unfortunately, this reconstitution may take weeks and not occur prior to the next scheduled chemotherapy cycle.

Erythropoietin, filgrastim, sargramostim, and oprelvekin have been approved by FDA to support oncology patients during specific types of cytopenias. These agents are generally associated with reconstitution of one specific lineage (e.g., myeloid, erythroid) and will not correct multilineage cytopenias. In an effort to facilitate a multilineage hematopoietic recovery, a number of hematopoietic stem cell stimulants are being investigated. These include stem cell factor, IL3-GMCSF fusion protein, and Flt3 receptor ligand. In many cases, the maximum tolerated dose of these agents is below that needed for clinical effectiveness of the compounds.

Angiotensin II (Ang-II) is traditionally recognized as a regulator of blood pressure. Recent preclinical studies show that Ang-II is also a potent regulator of tissue regeneration and can act as a hematopoietic factor [18,

26, 31, 32]. Ang-II and the A(1-7) analog have been shown to regulate the sensitivity of cells to growth factors. Mitigation of pressor activity from the parent compound, Ang-II, by deletion of the eighth amino acid reduces the risk of any A(1-7) adverse hypertensive properties. Ang-II and A(1-7) elevate the expression of growth factor such as platelet-derived growth factor, vascular endothelial cell growth factor, epidermal growth factor, and interleukin-6 [3, 19, 21, 25]. Addition of Ang-II to cell cultures with growth factors produces an additive effect on cell function [2, 8, 38]. This may be due to an increase in growth factor receptors, the expression of additional growth factors in response to the angiotensin peptides, or an effect of the angiotensin peptides independent of growth factor effects [17, 28, 36, 38].

Data derived from preclinical studies demonstrates the effectiveness of A(1-7) in accelerating hematopoietic recovery following both whole body radiation and chemotherapy-induced myelosuppression. Additional activity on bone marrow, including peripheral blood progenitor mobilization and proliferation similar to those effects seen with Ang-II [35], was demonstrated with A(1-7). The pharmacologic effects appear to be multilineage. Both before and after myelosuppression, increases in the numbers of CFU-GEMM, CFU-GM, BFU-E, and CFU-Meg have been demonstrated with A(1-7) [7, 33, 34].

When A(1-7) was combined with concomitant Neupogen therapy, both the concentrations of bone marrow progenitors and peripheral WBC were increased [7]. The purpose of this study was to determine the maximum tolerated dose of A(1-7) given prior to chemotherapy as well as examine the biologic activity following three cycles of chemotherapy in breast cancer patients. A comparator group of patients treated with filgrastim were studied for comparative safety and activity.

Materials and methods

This Phase I/II study was a prospective, multicenter, open-label, dose-escalation study to determine the maximum tolerated dose (MTD) as well as the optimal biologic dose (OBD) of A(1-7) on recovery of various hematopoietic lineages to chemotherapy. The effects of A(1-7) were evaluated in patients with newly diagnosed breast cancer receiving doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 every 21 days for at least three cycles of adjuvant chemotherapy following surgical tumor reduction. A(1-7) is identified by the formula: H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-OH. A(1-7) was supplied to the clinical pharmacy as a sterile solution at concentrations of 0.25, 1.0, 5.0, and 10.0 mg/ml packaged in a 1-ml glass vial with a pH of 6.0 and osmolality adjusted with mannitol. A(1-7) was stable for up to 2 weeks at room temperature and was stored at refrigerated temperatures (2–8°C, 36–46°F). The sterile stock solution was maintained at 2–8°C. A filgrastim (Neupogen, Amgen, Thousand Oaks, CA, USA) comparator arm was used to compare safety and response variables and to assess the safety of co-administered A(1-7) and filgrastim.

Prechemotherapy studies

The protocol was approved by the IRB at each study center. Each patient signed an informed consent. Patients who were otherwise in good health, had not received prior chemotherapy, and demonstrated normal hematology received a subcutaneous injection, once daily, of A(1-7) for 7 days followed by a 1-week rest period prior to any chemotherapy (cycle 0) in the interval between tumor reduction and planned chemotherapy. Dose escalation within an individual patient was not permitted. Dose escalation proceeded based on the occurrence of dose-limiting toxicity (DLT). Five ascending doses of 2.5, 10, 50, 75, and 100 mcg/kg/day were evaluated in a dose group of three patients beginning with the lowest dose and toxicity was assessed over cycle 0. A dose-limiting toxicity was defined as the occurrence of a NCI-CTC grade 3–4 toxicity considered related to the study drug. If one episode of DLT was observed among the first three patients at a particular dose level, then three additional patients were treated at that dose level. If less than two of six patients treated at a dose level experience a similar DLT during cycle 0, dose escalation continued. If two similar DLTs occurred in two of six patients at a dose level during cycle 0, then further entry into that dose level was terminated. The MTD was the previous dose level. If the MTD was not reached, the OBD was established at a dose level based on a review of the response data for white cell populations as well as platelets and red blood cells.

Postchemotherapy studies

Following cycle 0, chemotherapy was administered on Day 1. A(1-7) was administered for at least 10 days, beginning 2 days after chemotherapy. Up to three chemotherapy cycles followed by A(1-7) were repeated every 21 days or as indicated by patient tolerance. Any patient that failed to achieve an absolute neutrophil count (ANC) > 1,500/μl by day 15 [13 days of A(1-7)] received a filgrastim rescue of 5.0 mcg/kg/day until the ANC > 1,500/μl. One patient was randomized to receive filgrastim 5.0 mcg/kg/day, dosed identically to A(1-7), for each dosing group that received A(1-7). These patients were used for comparison to A(1-7)-treated patients for safety and efficacy. Any patient in the A(1-7) dosing arm that experienced an episode of febrile neutropenia following chemotherapy received filgrastim of 5.0 mcg/kg/day in combination with A(1-7) for the remaining chemotherapy cycles.

The primary safety endpoints were the incidence and grade of toxicity experienced by each dose group (DLT), changes in biochemistry, hematology, urinalysis, physical findings, and adverse events. Efficacy endpoints were time to nadir, nadir, and hematologic recovery as defined by the frequency of gradable hematologic toxicity.

A complete medical history, comprehensive physical exam, tumor assessment, Karnofsky Performance Status, chest X-ray, electrocardiogram (ECG), multiple gated acquisition scan (MUGA), thyroid-stimulating hormone (TSH), free thyroxine (T4), prothrombin time (PT)/partial thromboplastin time (PTT), and urinalysis, was conducted on all patients at screening and following the study. Vital signs, hematology, and serum chemistry were obtained on days 1, 3, 5, 7, 9, 12, 15, and 21 of each cycle throughout the study and analyzed by a central laboratory (Covance Central Laboratory, Princeton, NJ, USA). Pharmacokinetic samples were determined in plasma samples over the 6 h following the first injection of study drug. Additional samples were obtained at days 1, 4, and 7 prior to daily dosing. Plasma samples were analyzed using a method previously described [20].

Statistical analysis

An intent to treat (ITT) analysis was performed on all safety data. Differences between A(1-7) and filgrastim arms in peak and nadirs during cycle 3 were analyzed using a *t* test (nonpaired). Changes in prechemotherapy hematology during cycle 0 were performed using a paired *t* test. The lack of statistical power to predict efficacy was acknowledged.

A minimum of 15 patients and as many as 30 patients in the A(1-7) dosage group were to have been enrolled to complete the dose escalation before restrictive toxicity was observed. The sample size of the dose escalation was consistent with sound clinical judgment, based on clinical considerations, and was not intended to provide predictive power for efficacy. Five patients [one per A(1-7) group] were enrolled to receive filgrastim following chemotherapy. The sample size was based on clinical rather than statistical considerations.

Results

Twenty-one patients were randomized (ITT) into the study between October 1999 and May 2000. Twenty patients completed the study; one patient withdrew from the study prior to completing dose escalation and was replaced. The patient withdrew due to personal choice and an inability to remain compliant with the treatment schedule. Fifteen patients received A(1-7) as per the protocol and five patients received filgrastim. Baseline and oncologic history were similar between the two treatment arms (Table 1).

Table 1 Baseline demographics and oncologic history of patients

Parameter	A(1-7) ($N = 16$)	Filgrastim ($N = 5$)
Age (years)		
Mean	51.4	54
SD	10.1	5.2
Min	31	48
Median	51.5	53
Max	67	62
Size of tumor (cm)		
Mean	2.4	2.4
SD	0.6	2.6
Min	1.5	0.8
Median	2.4	1.6
Max	3.5	7
Race		
Caucasian	16	5
Menopausal status		
Pre	8	1
Post	8	4
Stage of disease		
Stage 1	4	0
Stage 2	11	4
Stage 3a	1	1

Continuous data are presented as mean ± standard deviation, median, minimum, and maximum observation. *N* Total number of patients in a group

Primary objective

The primary objective was to determine the MTD as well as the OBD of A(1-7) administered in a range from 2.5 to 100 mcg/kg/day before chemotherapy. Prior to chemotherapy, no dose-limiting toxicity was observed following A(1-7). After chemotherapy, the occurrence of adverse events was slightly lower in A(1-7) than filgrastim-treated patients.

Optimal biologic dose

The dose of A(1-7) necessary to minimize or mitigate toxicity varied by hematologic cell lineage during this study. Prevention of grade 2 or greater thrombocytopenia occurred at all doses of A(1-7). At A(1-7) doses greater than 10 mcg/kg/day, anemia (Hgb < 10 gm/dl) did not occur in 11 of 12 patients. At A(1-7) doses of > 10 mcg/kg, lymphopenia (< 500 cells/μl) did not occur in 9 of 12 patients. At A(1-7) doses of 10–100 mcg/kg, grade 4 toxicity of leukocytes was observed in only 1 of 12 patients. An evaluation of A(1-7)'s effects on the observation of hematologic toxicity across all cell lines showed the OBD to be 100 mcg/kg/day due to the lower frequency of grade 4 neutropenia at this dose.

Frequency of toxicity for hematologic parameters

The occurrence of grade 2 (moderate), grade 3 (severe), and grade 4 (life threatening) toxicity was evaluated over the course of the study. The frequency of patients

experiencing at least one episode of gradable toxicity during any cycle was compared between the treatment arms and dosage groups. Data for the frequency of toxicity are provided in Table 2. No patients treated with A(1-7) experienced any grade 2 or greater thrombocytopenia. There were no episodes of grade 3 or 4 anemia in any A(1-7)-treated patients. Grade 2 lymphopenia occurred in all patients. The incidence of grade 3 leukopenia was similar between filgrastim and A(1-7)-treated patients. By the third cycle, the incidence of patients with grade 4 neutropenia was similar between filgrastim 2/5 (40%) and A(1-7) 6/15 (40%) treated patients (data not shown). The absolute nadirs over the three chemotherapy cycles are provided in Table 3. By the third cycle, mean platelet nadirs were significantly higher ($P < 0.012$) with A(1-7) than filgrastim. The preservation of platelet counts was not dose dependent in A(1-7)-treated patients.

Time to nadir for hematologic parameters

The time to reach nadir was evaluated within each cycle of chemotherapy. The median times to nadir for platelets, hemoglobin, and lymphocytes were similar in all cycles for filgrastim and A(1-7)-treated patients. The median time to nadir for leukocytes was shorter for filgrastim patients than A(1-7) patients in cycle 1 (9 days vs. 14 days), in cycle 2 (9 days vs. 12 days), and in cycle 3 (9 days vs. 12 days). The median time to nadir for ANC was shorter in filgrastim patients than A(1-7) patients in cycle 1 (9 days vs. 15 days), in cycle 2 (9 days vs. 13 days), and in cycle 3 (9 days vs. 15 days).

Cumulative duration of toxicity

The mean cumulative (over three cycles) duration of platelets ≥ grade 2 was shorter following A(1-7) treatment (0 day) compared to filgrastim treatment (10.4 ± 13.7 days). Similarly, the cumulative duration of toxicity over the study interval for hemoglobin ≥ grade 2 was lower in A(1-7) patients than filgrastim patients (4.0 ± 7.9 days vs. 11.4 ± 10.2 days, respectively). There was a longer cumulative duration of lymphocytopenia ≥ grade 3 between A(1-7) (6.9 ± 11.4 days) and filgrastim (4.0 ± 3.0 days).

Cycle delays

All filgrastim-treated patients received chemotherapy on cycle throughout the study without dose reduction. All A(1-7)-treated patients received chemotherapy without dose reduction, although the use of a filgrastim rescue in 11 of the patients, given only due to protocol requirements, might have influenced this observation. One A(1-7)-treated patient had cycle delays following the first and second cycle related not to toxicity but for reasons related to patient preference.

Table 2 Frequency of the toxicity graded by NCI common toxicity criteria observed during the study

Parameter	Treatment group	*N*	Grade 2–4	Grade 3–4	Grade 4
Platelets	A(1-7) 2.5 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 10 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 50 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 75 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 100 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	Filgrastim	5	3/5 (60%)	1/5 (20%)	1/5 (20%)
Hemoglobin	A(1-7) 2.5 μg/kg	3	2/3 (66%)	0/3 (0%)	0/3 (0%)
	A(1-7) 10 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 50 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 75 μg/kg	3	0/3 (0%)	0/3 (0%)	0/3 (0%)
	A(1-7) 100 μg/kg	3	1/3 (33%)	0/3 (0%)	0/3 (0%)
	Filgrastim	5	3/5 (60%)	1/5 (20%)	0/5 (0%)
Lymphocytes	A(1-7) 2.5 μg/kg	3	3/3 (100%)	2/3 (66%)	NA
	A(1-7) 10 μg/kg	3	3/3 (100%)	1/3 (33%)	NA
	A(1-7) 50 μg/kg	3	3/3 (100%)	1/3 (33%)	NA
	A(1-7) 75 μg/kg	3	3/3 (100%)	0/3 (0%)	NA
	A(1-7) 100 μg/kg	3	3/3 (100%)	1/3 (33%)	NA
	Filgrastim	5	5/5 (100%)	4/5 (80%)	NA
Leukocytes	A(1-7) 2.5 μg/kg	3	3/3 (100%)	2/3 (66%)	2/3 (66%)
	A(1-7) 10 μg/kg	3	3/3 (100%)	2/3 (66%)	0/3 (0%)
	A(1-7) 50 μg/kg	3	3/3 (100%)	2/3 (66%)	1/3 (33%)
	A(1-7) 75 μg/kg	3	3/3 (100%)	3/3 (100%)	0/3 (0%)
	A(1-7) 100 μg/kg	3	3/3 (100%)	1/3 (33%)	0/3 (0%)
	Filgrastim	5	3/5 (60%)	3/5 (60%)	2/5 (40%)
ANC	A(1-7) 2.5 μg/kg	3	3/3 (100%)	3/3 (100%)	3/3 (100%)
	A(1-7) 10 μg/kg	3	3/3 (100%)	3/3 (100%)	2/3 (66%)
	A(1-7) 50 μg/kg	3	3/3 (100%)	3/3 (100%)	2/3 (66%)
	A(1-7) 75 μg/kg	3	3/3 (100%)	3/3 (100%)	2/3 (66%)
	A(1-7) 100 μg/kg	3	3/3 (100%)	3/3 (100%)	1/3 (33%)
	Filgrastim	5	3/5(60%)	2/5 (40%)	2/5 (40%)

Data are represented as the percentage of patients that experience at least 1 day of toxicity during any of the three cycles of chemotherapy classified by NCI Common Toxicity Criteria. *N* Number of patients in a group. Patients with grade 4 toxicity are counted in the grouping of grade 3–4 and in the grouping of grade 2–4. Patients with grade 3 toxicity are counted in the grouping of grade 3–4 and in the grouping of grade 2–4

Table 3 Nadir of hematologic measures during chemotherapy

Parameter	Treatment group	N	Cycle 1	Cycle 2	Cycle 3
Platelets	A(1-7) 2.5 μg/kg	3	184.7 ± 25.7	145.0 ± 37.3	153.7 ± 29.6
	A(1-7) 10 μg/kg	3	194.3 ± 22.7	198.0 ± 9.5	224.3 ± 37.1
	A(1-7) 50 μg/kg	3	249.0 ± 112.5	189.7 ± 54.2	207.7 ± 23.8
	A(1-7) 75 μg/kg	3	188.0 ± 23.6	181.3 ± 61.6	153.3 ± 36.4
	A(1-7) 100 μg/kg	3	163.6 ± 28.9	149.3 ± 23.9	159.0 ± 28.8
	Filgrastim	5	116.8 ± 47.5	111.4 ± 56.6	92.6 ± 49.6
Hemoglobin	A(1-7) 2.5 μg/kg	3	11.2 ± 1.44	9.7 ± 1.45	10.2 ± 1.23
	A(1-7) 10 μg/kg	3	11.9 ± 0.17	11.0 ± 0.20	10.8 ± 0.31
	A(1-7) 50 μg/kg	3	11.3 ± 0.76	10.7 ± 0.53	10.5 ± 0.15
	A(1-7) 75 μg/kg	3	11.7 ± 0.72	11.4 ± 0.21	10.9 ± 0.57
	A(1-7) 100 μg/kg	3	12.5 ± 1.36	11.7 ± 1.80	10.8 ± 1.93
	Filgrastim	5	11.6 ± 1.01	10.8 ± 1.50	9.7 ± 1.43
Lymphocytes	A(1-7) 2.5 μg/kg	3	0.91 ± 0.27	0.67 ± 0.39	0.46 ± 0.35
	A(1-7) 10 μg/kg	3	0.81 ± 0.06	0.72 ± 0.18	0.66 ± 0.21
	A(1-7) 50 μg/kg	3	0.67 ± 0.26	0.51 ± 0.28	0.56 ± 0.34
	A(1-7) 75 μg/kg	3	1.03 ± 0.10	0.88 ± 0.25	0.63 ± 0.11
	Av 100 μg/kg	3	0.99 ± 0.25	0.65 ± 0.41	0.65 ± 0.15
	Filgrastim	5	1.03 ± 0.42	0.84 ± 0.25	0.51 ± 0.21
Leukocytes	A(1-7) 2.5 μg/kg	3	1.83 ± 0.71	1.53 ± 0.84	1.20 ± 0.78
	A(1-7) 10 μg/kg	3	2.11 ± 0.71	2.32 ± 0.62	2.26 ± 0.78
	A(1-7) 50 μg/kg	3	2.17 ± 1.53	1.31 ± 0.73	1.45 ± 0.61
	A(1-7) 75 μg/kg	3	2.27 ± 0.86	2.30 ± 0.98	1.59 ± 0.29
	A(1-7) 100 μg/kg	3	2.45 ± 0.85	2.04 ± 0.94	1.82 ± 0.54
	Filgrastim	5	3.52 ± 2.13	3.09 ± 2.07	2.57 ± 2.22
ANC	A(1-7) 2.5 μg/kg	3	0.36 ± 0.06	0.41 ± 0.23	0.40 ± 0.14
	A(1-7) 10 μg/kg	3	0.41 ± 0.05	0.98 ± 0.57	0.98 ± 0.29
	A(1-7) 50 μg/kg	3	1.08 ± 1.05	0.55 ± 0.50	0.52 ± 0.19
	A(1-7) 75 μg/kg	3	0.87 ± 0.76	0.77 ± 0.72	0.59 ± 0.35
	A(1-7) 100 μg/kg	3	0.55 ± 0.19	0.71 ± 0.47	0.58 ± 0.19
	Filgrastim	5	1.82 ± 1.55	1.91 ± 1.54	1.71 ± 1.78

Data are represented as mean ± SD for the lowest plasma concentration of the specific parameter observed during each cycle of chemotherapy. Data were compared during cycle 3 using a *T* test corrected for equal variances. Platelets are listed as 10^3 cells/μl, Hemoglobin is listed as gm/dl, Lymphocytes are listed as 10^3 cells/μl, Leukocytes are listed as 10^3 cells/μl, and Absolute neutrophils are listed as 10^3 cells/μl

Episodes of febrile neutropenia

There was a single event of febrile neutropenia during the study, which occurred during A(1-7) treatment (2.5 mcg/kg/day) in cycle 2 at day 15. The event lasted for 3 days and there was no evidence of a bacterial infection and blood cultures to be negative.

Pharmacokinetics

The concentration of A(1-7) was determined in plasma samples over the 6 h following the first injection of study drug. Additional samples were obtained at days 1, 4, and 7 prior to dosing. Data for patient 602 was excluded because samples exceeded the range of the assay and dilution failed to correct the error in measurement (Table 4). Pharmacokinetic estimates at 2.5 mcg/kg appear to have poor reliability due to the small increase in plasma A(1-7) over endogenous levels. Excluding data from the 2.5 mcg/kg dose, the mean plasma half-life is 0.49 h (29 min) (range 0.32–0.86 h) and a volume of distribution of 3.71 l/kg (range 0.43–12.7). The relationship between dose and C_{max} was correlated and fit the regression line of:

$$C_{max} = 29.73 \times (\text{dose}) - 17.24 \, (r^2 = 0.96)$$

And for AUC with the equation:

$$AUC_{0-24} = 32.89 \times (\text{dose}) + 359.28 \, (r^2 = 0.87)$$

The relationship of dose to AUC and C_{max} indicates first-order plasma elimination kinetics. The comparison of pharmacokinetics to hematologic pharmacodynamics indicates that doses of ≥50 mcg/kg/day appear to be optimized for the majority of hematologic parameters.

Summary of safety

A filgrastim rescue starting on day 15 was required in 11/15 of the A(1-7)-treated patients at least during one cycle. In all but one case, the filgrastim rescue was a protocol requirement (ANC < 1,500 cells/μl) and was

Table 4 Derived pharmacokinetic estimates

Dose mcg/kg/day	AUC_{0-24} pg/ml/h	$T_{1/2}$ h^{-1}	C_{max} pg/ml	C_{max} range pg/ml	Volume of distribution (l/kg)
2.5	610.88	2.36	49.43	35–66	1.22
10	945.69	0.69	505.4	290–795	3.23
50	2,422.67	0.44	1,161.93	516–1,994	3.47
75	3,701.91	0.45	2,052.03	902–3,885	8.29
100	2,570.70	0.39	3,209.50	1,319–5,100	1.40

Derived mean pharmacokinetic data for A(1-7) by dose. Serum data were fitted using NONLIN modeling with a single compartment. *AUC* Area under the time concentration cure from 0 to 24 h, $T_{1/2}$ Extrapolated from 1 to 24 h, C_{max} The maximum concentration in pg/ml, and volume of distribution is estimated in l/kg

Table 5 Adverse events observed during the study

Provides a listing of adverse events (number of events, percent of patients experiencing an event) categorized by body system and relationship to study drug for A(1-7) and filgrastim treatment

Body system	A(1-7) treatment		Filgrastim treatment	
	Not related (%)	Related (%)	Not related (%)	Related (%)
Body	54 (96.4)	2 (3.6)	14 (87.5)	2 (12.5)
Cardiovascular	2 (100)	0	1 (100)	0
Digestive	81 (100)	0	23 (92)	2 (8)
Hematologic	20 (100)	0	18 (100)	0
Metabolic	4 (100)	0	4 (100)	0
Musculoskeletal	8 (50)	8 (50)	3 (75)	1 (25)
Nervous	7 (100)	0	6 (100)	0
Respiratory	18 (100)	0	4 (100)	0
Skin	16 (100)	0	7 (100)	0
Special Senses	9 (100)	0	2 (100)	0
Urogenital	4 (100)	0	0	0

not necessitated by an event of febrile neutropenia. Six of fifteen (40%) patients treated with A(1-7) experienced 13 adverse events prior to chemotherapy (cycle 0). Over the entire study, the average number of adverse events per patient was 14.6 (233 events in 16 patients) following A(1-7) of which 10 (4.3%) were drug-related (Table 5). There were three serious adverse events during the study [one A(1-7) patient experienced neutropenic fever, one filgrastim patient developed leukopenia, and one developed a problem at the injection site]. The frequency of stomatitis was lower in A(1-7) patients (40%) than in filgrastim patients (60%). Evaluations for changes from baseline to the lowest and highest-observed serum chemistry value were reviewed and no clinically significant changes were observed. No clinically meaningful changes in systolic, diastolic, or mean arterial pressure were observed in the 6 h following the first dose of A(1-7). Shift analysis of pre- to post-ECG, chest X-rays, and MUGA scans indicates there were no clinically meaningful changes following either A(1-7) or filgrastim administration. No change from baseline in serum-free T_4 levels or TSH levels were observed following filgrastim treatment. One A(1-7)-treated patient experienced a shift from low to normal in serum-free T_4 level and TSH. There were no clinically meaningful changes in urine casts, protein, cells, specific gravity, or pH over the study interval in any patient.

Prechemotherapy hematology

Baseline platelet, hemoglobin, lymphocytes, leukocytes, and absolute neutrophils were not different between the treatment arms. Pre- to postchanges in hematologic parameters were analyzed after seven daily injections of A(1-7) followed by a 7-day washout prior to chemotherapy (cycle 0). A(1-7) did not lead to any significant pre- to postchange in platelet count (pre- to post-: 260 ± 56.8 vs. $278 \pm 66.5 \times 10^3$ cells/μl), leukocytes (pre- to post-: 6.9 ± 1.4 vs. $7.3 \pm 2.0 \times 10^3$ cells/μl), or neutrophils (pre- to post-: 4.4 ± 1.1 vs. $4.5 \pm 1.4 \times 10^3$ cells/μl). A(1-7) led to a significant pre- to postincrease in hemoglobin (pre- to post-: 12.75 ± 1.15 vs. 13.23 ± 1.19 gm/dl, $P < 0.037$) and lymphocytes (pre to post: 1.84 ± 0.38 vs. $2.16 \pm 0.70 \times 10^3$ cells/μl, $P < 0.022$).

Postchemotherapy hematology

Based on a review of dose toxicity, the frequency of gradable toxicity was not different between dosages of 10 and 100 mcg/kg (with the exception for neutropenia at 100 mcg/kg); therefore, data from patients receiving A(1-7) doses between 10 and 100 mcg/kg were combined into a group ($N = 12$) to provide for a more robust comparison to filgrastim-treated patients ($N = 5$).

Over the course of the study, A(1-7)-treated patients demonstrated less variability in platelet concentrations over each cycle of chemotherapy compared to filgrastim-treated patients (Fig. 1). The third cycle recovery of platelets was not different between the treatment arms. The third cycle hemoglobin recovery was significantly different between A(1-7) (12.04 ± 0.31 gm/dl) and filgrastim (11.2 ± 0.87 gm/dl) ($P < 0.05$) (Fig. 2). By the third cycle, lymphocyte recovery was significantly different between A(1-7) ($1.28 \pm 0.09 \times 10^3$ cells/μl) and filgrastim ($0.78 \pm 0.05 \times 10^3$ cells/μl) ($P < 0.002$) (Fig. 3). By the third cycle, leukocyte recovery was significantly different between A(1-7) patients ($7.62 \pm 0.71 \times 10^3$ cells/μl) and filgrastim patients ($5.06 \pm 0.45 \times 10^3$ cells/μl) ($P < 0.05$) (Fig. 4). Following the third cycle, neutrophil recovery was significantly greater in A(1-7) patients ($5.58 \pm 0.61 \times 10^3$ cells/μl) than filgrastim patients ($3.98 \pm 0.37 \times 10^3$ cells/μl) ($P < 0.05$) (Fig. 5).

Discussion

This study assessed the safety and activity of A(1-7) following chemotherapy in patients with breast cancer. No dose-limiting toxicity was observed in the 14 days prior to chemotherapy. Doxorubicin/cyclophosphamide are well tolerated and commonly used first line regimens of chemotherapy in this patient group. As a result, they were chosen for this initial clinical trial with A(1-7).

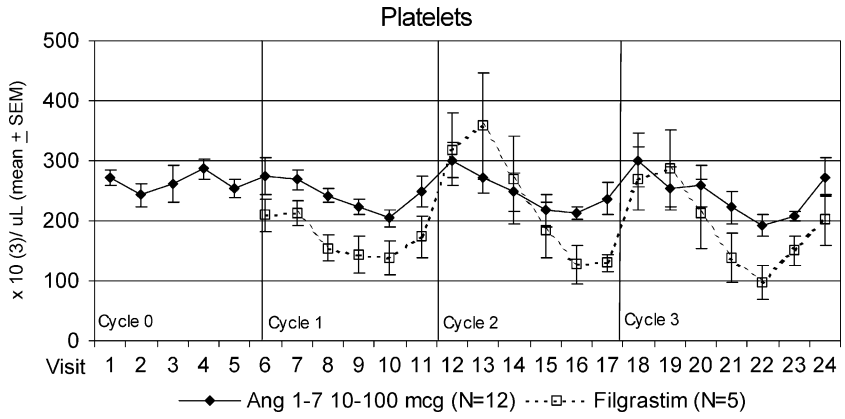

Fig. 1 Concentration of platelets observed over the study. Data are displayed as mean ± SEM for patients receiving doses from 10 to 100 mcg/kg. Cycle 0 is the treatment 14 days prior to the first chemotherapy administration. Cycles are 21 days in length. Visit 6, 12, and 18 represent prechemotherapy concentrations at the start of cycle 1, 2 and 3, respectively. Visit 7, 13, and 19 occurred 3 days after chemotherapy administration. Visit 8, 14, and 20 occurred 7 days after chemotherapy administration. Visit 9, 15, and 21 occurred 10 days after chemotherapy administration. Visit 10, 16, and 22 occurred 12 days after chemotherapy administration. Visit 11, 17, and 23 occurred 15 days after chemotherapy administration

Although apparent differences in lineage sensitivity to A(1-7) were seen, all responded to 100 mcg/kg, the OBD. At this dose, A(1-7) reduced the frequency and severity of thrombocytopenia, anemia, and lymphopenia compared to filgrastim. In the third cycle, absolute platelet nadirs were superior following A(1-7). As this study was not sufficiently powered to statistically assess differences in treatment response, larger follow-up studies are required.

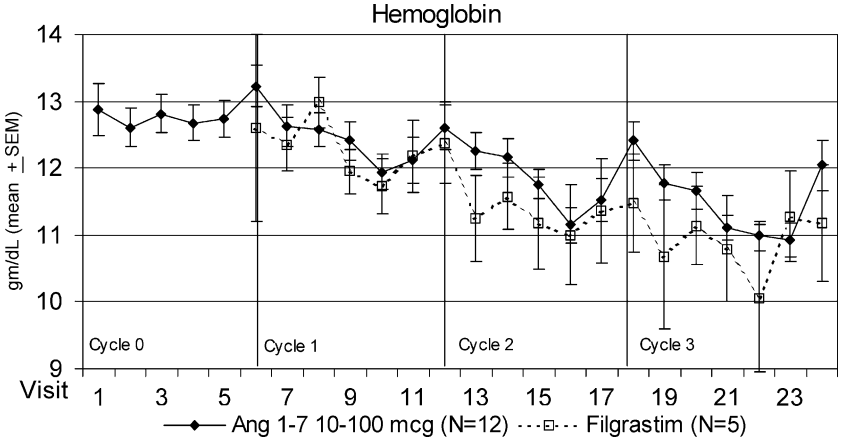

Fig. 2 Concentration of hemoglobin observed over the study. Data are displayed as mean ± SEM for patients receiving doses from 10 to 100 mcg/kg

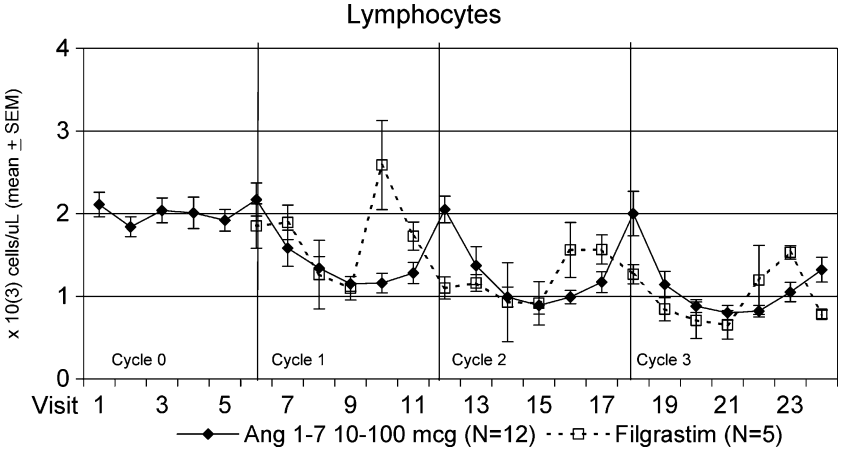

Fig. 3 Concentration of lymphocytes observed over the study. Data are displayed as mean ± SEM for patients receiving doses from 10 to 100 mcg/kg

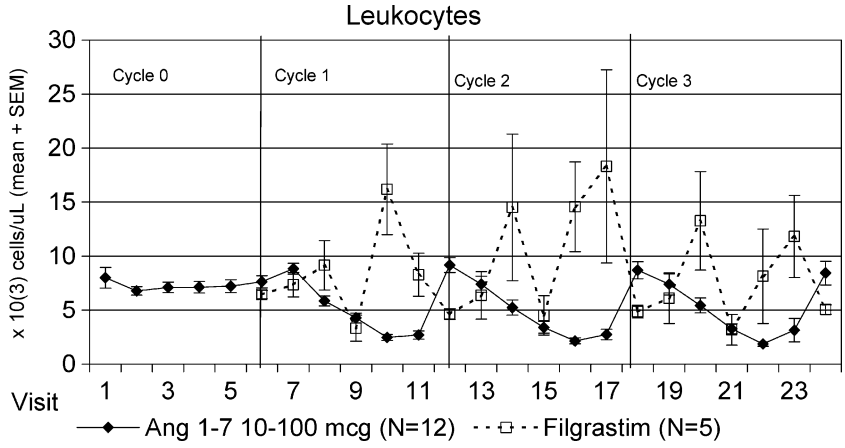

Fig. 4 Concentration of leukocytes observed over the study. Data are displayed as mean ± SEM for patients receiving doses from 10 to 100 mcg/kg

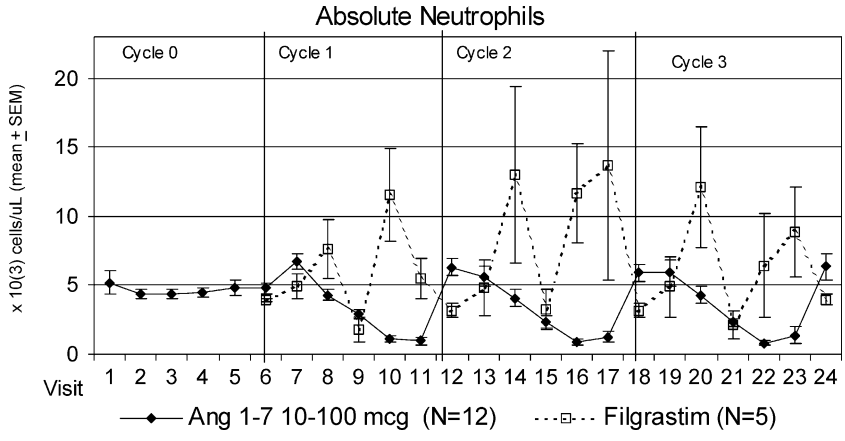

Fig. 5 Concentration of neutrophils observed over the study. Data are displayed as mean ± SEM for patients receiving doses from 10 to 100 mcg/kg

The effect of angiotensin on erythropoiesis has been well characterized. A number of studies, dating back to the 1960's, demonstrate the effect of Ang-II in erythropoiesis [12, 23, 27]. Ang-II was shown to increase proliferation of BFU-E from CD34+ cells in culture [26, 35]. Mrug et al. [26] reported that Ang-II increased the colony formation of erythroid progenitors. This increase could be mitigated by blocking the angiotensin type 1 receptor with losartan. In a study by Rodgers et al. [35], the ability of Ang-II to increase proliferation of BFU-E and other hematopoietic progenitor cells from both human cord blood isolated CD34+ cells and murine bone marrow were evaluated. In a human study, healthy volunteers were given an artificial hemorrhage of 750 ml, followed by an infusion of Ang-II, with and without losartan and captopril [11]. Angiotensin increased the C_{max} and AUC for endogenous erythropoietin by 67 and 40%, respectively, compared to a saline control. The effect of Ang-II on erythropoietin release was blocked by losartan, but not affected by captopril. The in vivo effects of angiotensin on erythropoiesis were further elucidated in a recent publication in ACE deficient mice [4]. Two strains of ACE knockout mice were shown to have a normocytic anemia associated with elevated plasma erythropoietin levels. Cr^{51} labeling of red cells showed that the knockout mice had a normal total blood volume but a reduced red cell mass. ACE knockout mice, which lack tissue ACE, were anemic despite having normal renal function and low plasma levels of Ang-II. Infusion of Ang-II for 2 weeks increased hematocrit to near normal levels. The authors concluded the anemia was present in spite of elevated erythropoietin levels and required Ang-II to facilitate normalization of hemoglobin concentrations.

Some of the effects of Ang-II on cell function may be mediated through alterations in growth factor production [14, 22, 30]. Ang-II has also been shown to regulate the sensitivity of cells to growth factors. Ang-II was shown to elevate the expression of receptors for growth factor such as vascular endothelial cell growth factor and EGF [3, 6]. This may be due to an increase in number of growth factor receptors, through expression of additional growth factors, in response to A-II or an effect of A-II independent of growth factor effects [17, 38]. In direct support of the effect of Ang II on bone marrow, an increase in the proliferation of colonies of GEMM, GM, MEG, and BFU-E were observed from both human CD34+ cord blood cells as well as murine bone marrow [7, 33, 34, 35].

A number of studies demonstrated a receptor-mediated effect of angiotensin in hematopoiesis. Direct measures of mRNA for angiotensin type 1 receptor were

reported in culture of marrow progenitors from animals and humans [26, 35]. The receptors were observed on stromal cells as well. In several reports, the angiotensin type 1 receptor blocker losartan blocked the proliferative or erythropoietic effects of angiotensin [10, 11, 26]. In addition, there are numerous reports of the effect of angiotensin receptor antagonist in the treatment of posttransplantation erythrocytosis [5].

The use of A(1-7) in this study demonstrated that daily doses of 2.5–100 mcg/kg were safe and well tolerated. No dose-limiting toxicity was observed, while an optimal biological dose was demonstrated. These studies warrant larger clinical trials in patients undergoing a greater number of treatment cycles at an A(1-7) dose of 100 mcg/kg/day with antineoplastic drugs of increased myelosuppressive toxicity.

Acknowledgement The authors would like to acknowledge the editorial assistance of Laura dizerega in the development of this paper.

References

1. Belt RJ, Leite C, Haas CD, Stepheans RL (1978) Incidence of hemorrhagic complications in patients with cancer. JAMA 239:2571–2574
2. Campbell-Boswell M, Robertson AL (1981) Effects of angiotensin II and vasopressin on human smooth muscle cells in vitro. Exp Mol Pathol 35:265–276
3. Chua CC, Hamdy RC, Chua BH (1998) Upregulation of vascular endothelial growth factor by angiotensin II in rat heart endothelial cells. Biochem Biophys Acta 1401:187–194
4. Cole J, Ertoy D, Lin H, Sutliff RL, Ezan E, Guyene TT, Capecchi M, Corvol P, Bernstein KE (2000) Lack of angiotensin II-facilitated erythropoiesis causes anemia in angiotensin-converting enzyme-deficient mice. J Clin Invest 106:1391–1398
5. Ducloux D, Fournier V, Bresson-Vautrin C, Chalopin JM (1998) Long-term follow-up of renal transplant reciepients treated with losartan for post-transplant erythosis. Transpl Int 11:312–315
6. Eguchi S, Numaguchi K, Iwasaki H, Matsumoto T, Yamakawa T, Utsunomiya H, Motley ED, Kawakatsu H, Owada KM, Hirata Y et al (1998) Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen activated protein kinase activation in vascular smooth muscle cells. J Biol Chem 273:8890–8896
7. Ellefson DD, Espinoza T, Roda N, Maldonado S, Rodgers KE (2004) Synergistic effects on co-administration of Angiotensin 1–7 and Neupogen on hematopoietic recovery in mice. Cancer Chemother Pharmacol 53(1):15–24
8. Emmett N, Archibald E, Harris-Hooker S, Dukes L (1986) Effect of saralasin (angiotensin II antagonist) on 3T3 cell growth and proliferation. J Cell Biol 103 (Suppl) 171a
9. Fine JS, Cai XY, Justice L, Gommoll CP, Hamiltion LD, Waters TA, Narula SK, Bober LA, Grace MJ (1997) A specific stimulator of granulocyte colony-stimulating factor accelerates recovery from cyclophosphamide-induced neutropenia in the mouse. Blood 90:795–802
10. Freudenthaler SM, Lucht I, Schenk T, Brink M, Gleiter CH (2000) Dose-dependent effect of angiotensin II on human erythropoietin production. Pflugers Arch 439:838–844
11. Freudenthaler SM, Schreeb KH, Korner T, Gleiter CH (1999) Angiotensin II increases erythropoietin production in healthy volunteers. Eur J Clin Invest 29:816–823
12. Fried W, Barone-Varelas J, Morley C (1984) Factors that regulated extrarenal erythropoietin production. Blood Cells 10:287–304
13. Gardner RV, Astle CM, Harrison DE (1997) Hematopoietic precursor cell exhaustion is a cause of proliferative defect in primitive hematopoietic stem cells (PHSC) after chemotherapy. Exp Hematol 25:495–501
14. Gibbons GH, Pratt RE, Dzau VJ (1992) Vascular smooth muscle cell hypertrophy vs hyperplasia. Autocrine transforming growth factor-beta 1 expression determines growth response to angiotensin II. J Clin Invest 90:456–461
15. Glaspy J, Bukowski R, Steinberg D, Taylor C, Tchekmedyian S, Vadhan-Ray S (1997) Impact of therapy with Epoetin alpha on clinical outcomes in patients with nonmyeloid malignancies during cancer chemotherapy in community oncology practice. Procrit study group. J Clin Oncol 15:1218–1234
16. Haas R, Gericke G, Witt B, Cayeux S, Hunstein W (1993) Increased serum levels of granulocyte colony-stimulating factor after autologous bone marrow or stem cell transplantation. Exp Hematol 21(1):109–113
17. Higueruelo S, Romero R (1997) Angiotensin II requires PDGF-BB to induce DNA synthesis in rat mesangial cells cultured in an exogenous insulin-free medium. Nephrol Dial Transplant 12:694–700
18. Hsueh WA, Do YS, Anderson PW, Law RE (1995) Angiotensin II in cell growth and matrix production. Adv Exp Med Biol 377:217–223
19. Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ (1993) Multiple autocrine growth factors modulate vascular smooth muscle cell growth response to angiotensin II. J Clin Invest 91:2268–2274
20. Kohara K, Tabuchi Y, Senenayake P, Brosnihan KB, Ferrario CM (1991) Reassessment of plasma angiotensin measurement: effects of protease inhibitors and sample handling procedures. Peptides 12:1135–1141
21. Koibuchi Y, Lee WS, Gibbons GH, Pratt RE (1993) Role of transforming growth factor β-1 in the cellular growth response to angiotensin II. Hypertension 21:1046–1050
22. Mangiarua EI, Palmer VL, Lloyd LL, McCumbee WD (1997) Platelet-derived growth factor mediates angiotensin II-induced DNA synthesis in vascular smooth muscle cells. Arch Physiol Biochem 105:151–157
23. Mann DL, Donati RM, Gallagher NI (1966) Effect of renin, angiotensin II, and aldosterone on erythropoiesis. Proc Soc Exp Biol Med 121:1152–1154
24. Miller CB, Jones RJ, Piantadosi S, Abeloff MD, Spivak JL (1990) Decreased erythropoietin response in patients with anemia of cancer. NEJM 322:1689–1692
25. Moriyama T, Fujibayashi MF, Fujiwara Y, Kaneko T, Xia C, Imai E, Kamada T, Ando A, Ueda N (1995) Angiotensin II stimulates interleukin 6 release from cultured mouse mesangial cells. J Am Soc Nephrol 6:95–101
26. Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT (1997) Angiotensin II stimulates proliferation of normal erythroid progenitors. J Clin Invest 100:2310–2314
27. Nakao K, Shirakura T, Azuma M, Maekawa T (1967) Studies on erythropoietic action of angiotensin II. Blood 29:754–760
28. Owens GK (1985) Differential effects of antihypertensive drug therapy on vascular smooth muscle hypertrophy, hyperploidy and hyperplasia in the spontaneous hypertensive rat. Circ Res 56:525–536
29. Patchen ML, MacVittie TJ, Williams JL, Schwartz GN, Souza LM (1991) Administration of interleukin-6 stimulates multilineage hematopoiesis and accelerates recovery from radiation-induced hematopoietic depression. Blood 77:472–480
30. Peifely KA, Winkles JA (1998) Angiotensin II and endothelin-1 increase fibroblast growth factor-2 mRNA expression in vascular smooth muscle cells. Biochem Biophys Res Commun 242:202–208
31. Rodgers KE, Abiko M, Girgis W, St. Amand K, Campeau J, diZerega GS (1997a) Acceleration of dermal tissue repair by angiotensin II. Wound Repair Regen 5:175–183

32. Rodgers KE, DeCherney AH, St. Amand KM, Dougherty WR, Felix JC, Girgis WW, diZerega GS (1997b) Histologic alterations in dermal repair after thermal injury: effects of topical angiotensin II. Burn Care Rehabil 18:381–388
33. Rodgers KE, Xiong S, diZerega GS (2003) Effect of Angiotensin II and Angiotensin (1-7) on white blood cell recovery after intravenous chemotherapy. Cancer Chemother Pharmacol 51:97–106
34. Rodgers KE, Xiong S, diZerega GS (2002) Accelerated recovery from irradiation injury by Angiotensin II. Cancer Chemother Pharmacol 49:403–411
35. Rodgers KE, Xiong S, Steer R, diZerega GS (2000) Effects of angiotensin II on hematopoietic progenitor cell proliferation. Stem Cells 18:287–294
36. Schorb W, Conrad KM, Singer HA, Dostal DE, Baker KM (1995) Angiotensin II is a potent stimulator of MAP-kinase activity in neonatal rat cardiac fibroblasts. J Mol Cell Cardiol 27:1151–1160
37. Seidenfeld J, Piper M, Flamm C, Hasselbland V, Armitage JO, Bennett CL, Gordon MS, Lichtin AE, Wade JL, Wolf S, Aronson N et al (2001) Epoetin treatment of anemia associated with cancer therapy: a systematic review and meta-analysis on controlled clinical trials. J Natl Cancer Inst 93:1204–1214
38. Stouffer GA, Owensm GK (1992) Angiotensin II induced mitogenesis of spontaneously hypertensive rat derived cultured smooth muscle cells is dependent on autocrine production of transforming growth factor-β. Circ Res 70:820
39. Talcott JA, Finberg R, Mayer RJ, Goldman L (1988) The medical course of cancer patients with fever and neutropenia. Clinical identification of a low-risk subgroup at presentation. Arch Intern Med 148:2561–2568
40. Wathen LM, Knapp SA, DeGowin RL (1981) Suppression of marrow stromal cells and microenvironment damage following sequential radiation and cyclophosphamide. Int J Radiat Oncol Biol Phys 7:935–941